FIGURE 1

```
AGTCCCAGACGGGCTTTTCCCAGAGAGCTAAAAGAGAAGGGCCAGAGAATGTCGTCCCAG
CCAGCAGGGAACCAGACCTCCCCCGGGGCCACAGAGGACTACTCCTATGGCAGCTGGTAC
ATCGATGAGCCCCAGGGGGGCGAGGAGCTCCAGCCAGAGGGGGAAGTGCCCTCCTGCCAC
ACCAGCATACCACCCGGCCTGTACCACGCCTGCCTGGCCTCGCTGTCAATCCTTGTGCTG
CTGCTCCTGGCCATGCTGGTGAGGCGCCGCCAGCTCTGGCCTGACTGTGTGCGTGGCAGG
CCCGGCCTGCCCAGCCCTGTGGATTTCTTGGCTGGGGACAGGCCCCGGGCAGTGCCTGCT
GCTGTTTTCATGGTCCTCCTGAGCTCCCTGTGTTTGCTGCTCCCCGACGAGGACGCATTG
CCCTTCCTGACTCTCGCCTCAGCACCCAGCCAAGATGGGAAAACTGAGGCTCCAAGAGGG
GCCTGGAAGATACTGGGACTGTTCTATTATGCTGCCCTCTACTACCCTCTGGCTGCCTGT
GCCACGGCTGGCCACACAGCTGCACACCTGCTCGGCAGCACGCTGTCCTGGGCCCACCTT
GGGGTCCAGGTCTGGCAGAGGGCAGAGTGTCCCCAGGTGCCCAAGATCTACAAGTACTAC
TCCCTGCTGGCCTCCCTGCCTCTCCTGCTGGGCCTCGGATTCCTGAGCCTTTGGTACCCT
GTGCAGCTGGTGAGAAGCTTCAGCCGTAGGACAGGAGCAGGCTCCAAGGGGCTGCAGAGC
AGCTACTCTGAGGAATATCTGAGGAACCTCCTTTGCAGGAAGAAGCTGGGAAGCAGCTAC
CACACCTCCAAGCATGGCTTCCTGTCCTGGGCCCGCGTCTGCTTGAGACACTGCATCTAC
ACTCCACAGCCAGGATTCCATCTCCCGCTGAAGCTGGTGCTTTCAGCTACACTGACAGGG
ACGGCCATTTACCAGGTGGCCCTGCTGCTGCTGGTGGGCGTGGTACCCACTATCCAGAAG
GTGAGGGCAGGGGTCACCACGGATGTCTCCTACCTGCTGGCCGGCTTTGGAATCGTGCTC
TCCGAGGACAAGCAGGAGGTGGTGGAGCTGGTGAAGCACCATCTGTGGGCTCTGGAAGTG
TGCTACATCTCAGCCTTGGTCTTGTCCTGCTTACTCACCTTCCTGGTCCTGATGCGCTCA
CTGGTGACACACAGGACCAACCTTCGAGCTCTGCACCGAGGAGCTGCCCTGGACTTGAGT
CCCTTGCATCGGAGTCCCCATCCCTCCCGCCAAGCCATATTCTGTTGGATGAGCTTCAGT
GCCTACCAGACAGCCTTTATCTGCCTTGGGCTCCTGGTGCAGCAGATCATCTTCTTCCTG
GGAACCACGGCCCTGGCCTTCCTGGTGCTCATGCCTGTGCTCCATGGCAGGAACCTCCTG
CTCTTCCGTTCCCTGGAGTCCTCGTGGCCCTTCTGGCTGACTTTGGCCCTGGCTGTGATC
CTGCAGAACATGGCAGCCCATTGGGTCTTCCTGGAGACTCATGATGGACACCCACAGCTG
ACCAACCGGCGAGTGCTCTATGCAGCCACCTTTCTTCTCTTCCCCCTCAATGTGCTGGTG
GGTGCCATGGTGGCCACCTGGCGAGTGCTCCTCTCTGCCCTCTACAACGCCATCCACCTT
GGCCAGATGGACCTCAGCCTGCTGCCACCGAGAGCCGCCACTCTCGACCCCGGCTACTAC
ACGTACCGAAACTTCTTGAAGATTGAAGTCAGCCAGTCGCATCCAGCCATGACAGCCTTC
TGCTCCCTGCTCCTGCAAGCGCAGAGCCTCCTACCCAGGACCATGGCAGCCCCCCAGGAC
AGCCTCAGACCAGGGGAGGAAGACGAAGGGATGCAGCTGCTACAGACAAAGGACTCCATG
GCCAAGGGAGCTAGGCCCGGGGCCAGCCGCGGCAGGGCTCGCTGGGGTCTGGCCTACACG
CTGCTGCACAACCCAACCCTGCAGGTCTTCCGCAAGACGGCCCTGTTGGGTGCCAATGGT
GCCCAGCCCTGAGGGCAGGGAAGGTCAACCCACCTGCCCATCTGTGCTGAGGCATGTTCC
TGCCTACCATCCTCCTCCCTCCCCGGCTCTCCTCCCAGCATCACACCAGCCATGCAGCCA
GCAGGTCCTCCGGATCACTGTGGTTGGGTGGAGGTCTGTCTGCACTGGGAGCCTCAGGAG
GGCTCTGCTCCACCCACTTGGCTATGGGAGAGCCAGCAGGGGTTCTGGAGAAAAAAACTG
GTGGGTTAGGGCCTTGGTCCAGGAGCCAGTTGAGCCAGGGCAGCCACATCCAGGCGTCTC
CCTACCCTGGCTCTGCCATCAGCCTTGAAGGGCCTCGATGAAGCCTTCTCTGGAACCACT
CCAGCCCAGCTCCACCTCAGCCTTGGCCTTCACGCTGTGGAAGCAGCCAAGGCACTTCCT
CACCCCCTCAGCGCCACGGACCTCTCTGGGGAGTGGCCGGAAAGCTCCCGGTCCTCTGGC
CTGCAGGGCAGCCCAAGTCATGACTCAGACCAGGTCCCACACTGAGCTGCCCACACTCGA
GAGCCAGATATTTTTGTAGTTTTTATGCCTTTGGCTATTATGAAAGAGGTTAGTGTGTTC
CCTGCAATAAACTTGTTCCTGAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
```

FIGURE 2

```
MSSQPAGNQTSPGATEDYSYGSWYIDEPQGGEELQPEGEVPSCHTSIPPGLYHACLASLS
ILVLLLLAMLVRRRQLWPDCVRGRPGLPSPVDFLAGDRPRAVPAAVFMVLLSSLCLLLPD
EDALPFLTLASAPSQDGKTEAPRGAWKILGLFYYAALYYPLAACATAGHTAAHLLGSTLS
WAHLGVQVWQRAECPQVPKIYKYYSLLASLPLLLGLGFLSLWYPVQLVRSFSRRTGAGSK
GLQSSYSEEYLRNLLCRKKLGSSYHTSKHGFLSWARVCLRHCIYTPQPGFHLPLKLVLSA
TLTGTAIYQVALLLLVGVVPTIQKVRAGVTTDVSYLLAGFGIVLSEDKQEVVELVKHHLW
ALEVCYISALVLSCLLTFLVLMRSLVTHRTNLRALHRGAALDLSPLHRSPHPSRQAIFCW
MSFSAYQTAFICLGLLVQQIIFFLGTTALAFLVLMPVLHGRNLLLFRSLESSWPFWLTLA
LAVILQNMAAHWVFLETHDGHPQLTNRRVLYAATFLLFPLNVLVGAMVATWRVLLSALYN
AIHLGQMDLSLLPPRAATLDPGYYTYRNFLKIEVSQSHPAMTAFCSLLLQAQSLLPRTMA
APQDSLRPGEEDEGMQLLQTKDSMAKGARPGASRGRARWGLAYTLLHNPTLQVFRKTALL
GANGAQP
```

Important features of the protein:

Signal peptide:

None

Transmembrane domain:

54-69
102-119
148-166
207-222
301-320
364-380
431-451
474-489
560-535

Motif file:
Motif name: N-glycosylation site.

8-12

Motif name: N-myristoylation site.

50-56
176-182
241-247
317-323
341-347
525-531
627-633
631-637
640-646
661-667

Motif name: Prokaryotic membrane lipoprotein lipid attachment site.

364-375

Motif name: ATP/GTP-binding site motif A (P-loop).

132-140

FIGURE 3A

PRO	XXXXXXXXXXXXXXX	(Length = 15 amino acids)
Comparison Protein	XXXXXYYYYYYY	(Length = 12 amino acids)

% amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =

5 divided by 15 = 33.3%

FIGURE 3B

PRO	XXXXXXXXXX	(Length = 10 amino acids)
Comparison Protein	XXXXXYYYYYYZZYZ	(Length = 15 amino acids)

% amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =

5 divided by 10 = 50%

FIGURE 3C

PRO-DNA NNNNNNNNNNNNNN (Length = 14 nucleotides)

Comparison DNA NNNNNNLLLLLLLLLL (Length = 16 nucleotides)

% nucleic acid sequence identity =

(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =

6 divided by 14 = 42.9%

FIGURE 3D

PRO-DNA	NNNNNNNNNNNN	(Length = 12 nucleotides)
Comparison DNA	NNNNLLLVV	(Length = 9 nucleotides)

% nucleic acid sequence identity =

(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =

4 divided by 12 = 33.3%

FIGURE 4A

```
/*
 *
 * C-C increased from 12 to 15
 * Z is average of EQ
 * B is average of ND
 * match with stop is _M; stop-stop = 0; J (joker) match = 0
 */
#define   _M       -8          /* value of a match with a stop */

int       _day[26][26] = {
/*       A B C D E F G H I J K L M N O P Q R S T U V W X Y Z */
/* A */    { 2, 0,-2, 0, 0,-4, 1,-1,-1, 0,-1,-2,-1, 0,_M, 1, 0,-2, 1, 1, 0, 0,-6, 0,-3, 0},
/* B */    { 0, 3,-4, 3, 2,-5, 0, 1,-2, 0, 0,-3,-2, 2,_M,-1, 1, 0, 0, 0, 0,-2,-5, 0,-3, 1},
/* C */    {-2,-4,15,-5,-5,-4,-3,-3,-2, 0,-5,-6,-5,-4,_M,-3,-5,-4, 0,-2, 0,-2,-8, 0, 0,-5},
/* D */    { 0, 3,-5, 4, 3,-6, 1, 1,-2, 0, 0,-4,-3, 2,_M,-1, 2,-1, 0, 0, 0,-2,-7, 0,-4, 2},
/* E */    { 0, 2,-5, 3, 4,-5, 0, 1,-2, 0, 0,-3,-2, 1,_M,-1, 2,-1, 0, 0, 0,-2,-7, 0,-4, 3},
/* F */    {-4,-5,-4,-6,-5, 9,-5,-2, 1, 0,-5, 2, 0,-4,_M,-5,-5,-4,-3,-3, 0,-1, 0, 0, 7,-5},
/* G */    { 1, 0,-3, 1, 0,-5, 5,-2,-3, 0,-2,-4,-3, 0,_M,-1,-1,-3, 1, 0, 0,-1,-7, 0,-5, 0},
/* H */    {-1, 1,-3, 1, 1,-2,-2, 6,-2, 0, 0,-2,-2, 2,_M, 0, 3, 2,-1,-1, 0,-2,-3, 0, 0, 2},
/* I */    {-1,-2,-2,-2,-2, 1,-3,-2, 5, 0,-2, 2, 2,-2,_M,-2,-2,-2,-1, 0, 0, 4,-5, 0,-1,-2},
/* J */    { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,_M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* K */    {-1, 0,-5, 0, 0,-5,-2, 0,-2, 0, 5,-3, 0, 1,_M,-1, 1, 3, 0, 0, 0,-2,-3, 0,-4, 0},
/* L */    {-2,-3,-6,-4,-3, 2,-4,-2, 2, 0,-3, 6, 4,-3,_M,-3,-2,-3,-3,-1, 0, 2,-2, 0,-1,-2},
/* M */    {-1,-2,-5,-3,-2, 0,-3,-2, 2, 0, 0, 4, 6,-2,_M,-2,-1, 0,-2,-1, 0, 2,-4, 0,-2,-1},
/* N */    { 0, 2,-4, 2, 1,-4, 0, 2,-2, 0, 1,-3,-2, 2,_M,-1, 1, 0, 1, 0, 0,-2,-4, 0,-2, 1},
/* O */                                       {_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,
0,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M},
/* P */    { 1,-1,-3,-1,-1,-5,-1, 0,-2, 0,-1,-3,-2,-1,_M, 6, 0, 0, 1, 0, 0,-1,-6, 0,-5, 0},
/* Q */    { 0, 1,-5, 2, 2,-5,-1, 3,-2, 0, 1,-2,-1, 1,_M, 0, 4, 1,-1,-1, 0,-2,-5, 0,-4, 3},
/* R */    {-2, 0,-4,-1,-1,-4,-3, 2,-2, 0, 3,-3, 0, 0,_M, 0, 1, 6, 0,-1, 0,-2, 2, 0,-4, 0},
/* S */    { 1, 0, 0, 0, 0,-3, 1,-1,-1, 0, 0,-3,-2, 1,_M, 1,-1, 0, 2, 1, 0,-1,-2, 0,-3, 0},
/* T */    { 1, 0,-2, 0, 0,-3, 0,-1, 0, 0, 0,-1,-1, 0,_M, 0,-1,-1, 1, 3, 0, 0,-5, 0,-3, 0},
/* U */    { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,_M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* V */    { 0,-2,-2,-2,-2,-1,-1,-2, 4, 0,-2, 2, 2,-2,_M,-1,-2,-2,-1, 0, 0, 4,-6, 0,-2,-2},
/* W */    {-6,-5,-8,-7,-7, 0,-7,-3,-5, 0,-3,-2,-4,-4,_M,-6,-5, 2,-2,-5, 0,-6,17, 0, 0,-6},
/* X */    { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,_M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* Y */    {-3,-3, 0,-4,-4, 7,-5, 0,-1, 0,-4,-1,-2,-2,_M,-5,-4,-4,-3,-3, 0,-2, 0, 0,10,-4},
/* Z */    { 0, 1,-5, 2, 3,-5, 0, 2,-2, 0, 0,-2,-1, 1,_M, 0, 3, 0, 0, 0, 0,-2,-6, 0,-4, 4}
};
```

FIGURE 4B

```
/*
 */
#include <stdio.h>
#include <ctype.h>

#define  MAXJMP        16       /* max jumps in a diag */
#define  MAXGAP        24       /* don't continue to penalize gaps larger than this */
#define  JMPS          1024     /* max jmps in an path */
#define  MX            4        /* save if there's at least MX-1 bases since last jmp */

#define  DMAT          3        /* value of matching bases */
#define  DMIS          0        /* penalty for mismatched bases */
#define  DINS0         8        /* penalty for a gap */
#define  DINS1         1        /* penalty per base */
#define  PINS0         8        /* penalty for a gap */
#define  PINS1         4        /* penalty per residue */

struct jmp {
         short            n[MAXJMP];        /* size of jmp (neg for dely) */
         unsigned short   x[MAXJMP];        /* base no. of jmp in seq x */
};                                          /* limits seq to 2^16 -1 */

struct diag {
         int              score;            /* score at last jmp */
         long             offset;           /* offset of prev block */
         short            ijmp;             /* current jmp index */
         struct jmp       jp;               /* list of jmps */
};

struct path {
         int      spc;              /* number of leading spaces */
         short    n[JMPS];/* size of jmp (gap) */
         int      x[JMPS];/* loc of jmp (last elem before gap) */
};

char                 *ofile;                /* output file name */
char                 *namex[2];             /* seq names: getseqs() */
char                 *prog;                 /* prog name for err msgs */
char                 *seqx[2];              /* seqs: getseqs() */
int                  dmax;                  /* best diag: nw() */
int                  dmax0;                 /* final diag */
int                  dna;                   /* set if dna: main() */
int                  endgaps;               /* set if penalizing end gaps */
int                  gapx, gapy;            /* total gaps in seqs */
int                  len0, len1;            /* seq lens */
int                  ngapx, ngapy;          /* total size of gaps */
int                  smax;                  /* max score: nw() */
int                  *xbm;                  /* bitmap for matching */
long                 offset;                /* current offset in jmp file */
struct   diag        *dx;                   /* holds diagonals */
struct   path        pp[2];                 /* holds path for seqs */

char                 *calloc(), *malloc(), *index(), *strcpy();
char                 *getseq(), *g_calloc();
```

FIGURE 4C

```
/* Needleman-Wunsch alignment program
 *
 * usage: progs file1 file2
 *   where file1 and file2 are two dna or two protein sequences.
 *   The sequences can be in upper- or lower-case an may contain ambiguity
 *   Any lines beginning with ';', '>' or '<' are ignored
 *   Max file length is 65535 (limited by unsigned short x in the jmp struct)
 *   A sequence with 1/3 or more of its elements ACGTU is assumed to be DNA
 *   Output is in the file "align.out"
 *
 * The program may create a tmp file in /tmp to hold info about traceback.
 * Original version developed under BSD 4.3 on a vax 8650
 */
#include "nw.h"
#include "day.h"

static      _dbval[26] = {
            1,14,2,13,0,0,4,11,0,0,12,0,3,15,0,0,0,5,6,8,8,7,9,0,10,0
};

static      _pbval[26] = {
            1, 2|(1<<('D'-'A'))|(1<<('N'-'A')), 4, 8, 16, 32, 64,
            128, 256, 0xFFFFFFF, 1<<10, 1<<11, 1<<12, 1<<13, 1<<14,
            1<<15, 1<<16, 1<<17, 1<<18, 1<<19, 1<<20, 1<<21, 1<<22,
            1<<23, 1<<24, 1<<25|(1<<('E'-'A'))|(1<<('Q'-'A'))
};
```

main

```
main(ac, av)
            int         ac;
            char        *av[];
{
            prog = av[0];
            if (ac != 3) {
                        fprintf(stderr,"usage: %s file1 file2\n", prog);
                        fprintf(stderr,"where file1 and file2 are two dna or two protein sequences.\n");
                        fprintf(stderr,"The sequences can be in upper- or lower-case\n");
                        fprintf(stderr,"Any lines beginning with ';' or '<' are ignored\n");
                        fprintf(stderr,"Output is in the file \"align.out\"\n");
                        exit(1);
            }
            namex[0] = av[1];
            namex[1] = av[2];
            seqx[0] = getseq(namex[0], &len0);
            seqx[1] = getseq(namex[1], &len1);
            xbm = (dna)? _dbval : _pbval;

            endgaps = 0;                        /* 1 to penalize endgaps */
            ofile = "align.out";                /* output file */

            nw();               /* fill in the matrix, get the possible jmps */
            readjmps();         /* get the actual jmps */
            print();            /* print stats, alignment */

            cleanup(0);         /* unlink any tmp files */
}
```

FIGURE 4D

```
/* do the alignment, return best score: main()
 * dna: values in Fitch and Smith, PNAS, 80, 1382-1386, 1983
 * pro: PAM 250 values
 * When scores are equal, we prefer mismatches to any gap, prefer
 * a new gap to extending an ongoing gap, and prefer a gap in seqx
 * to a gap in seq y.
 */
nw()                                                                                    nw
{
        char            *px, *py;           /* seqs and ptrs */
        int             *ndely, *dely;      /* keep track of dely */
        int             ndelx, delx;        /* keep track of delx */
        int             *tmp;               /* for swapping row0, row1 */
        int             mis;                /* score for each type */
        int             ins0, ins1;         /* insertion penalties */
        register        id;                 /* diagonal index */
        register        ij;                 /* jmp index */
        register        *col0, *col1;       /* score for curr, last row */
        register        xx, yy;             /* index into seqs */

        dx = (struct diag *)g_calloc("to get diags", len0+len1+1, sizeof(struct diag));

        ndely = (int *)g_calloc("to get ndely", len1+1, sizeof(int));
        dely = (int *)g_calloc("to get dely", len1+1, sizeof(int));
        col0 = (int *)g_calloc("to get col0", len1+1, sizeof(int));
        col1 = (int *)g_calloc("to get col1", len1+1, sizeof(int));
        ins0 = (dna)? DINS0 : PINS0;
        ins1 = (dna)? DINS1 : PINS1;

        smax = -10000;
        if (endgaps) {
                for (col0[0] = dely[0] = -ins0, yy = 1; yy <= len1; yy++) {
                        col0[yy] = dely[yy] = col0[yy-1] - ins1;
                        ndely[yy] = yy;
                }
                col0[0] = 0;        /* Waterman Bull Math Biol 84 */
        }
        else
                for (yy = 1; yy <= len1; yy++)
                        dely[yy] = -ins0;

        /* fill in match matrix
         */
        for (px = seqx[0], xx = 1; xx <= len0; px++, xx++) {
                /* initialize first entry in col
                 */
                if (endgaps) {
                        if (xx == 1)
                                col1[0] = delx = -(ins0+ins1);
                        else
                                col1[0] = delx = col0[0] - ins1;
                        ndelx = xx;
                }
                else {
                        col1[0] = 0;
                        delx = -ins0;
                        ndelx = 0;
                }
```

FIGURE 4E

```
for (py = seqx[1], yy = 1; yy <= len1; py++, yy++) {
        mis = col0[yy-1];
        if (dna)
                mis += (xbm[*px-'A']&xbm[*py-'A'])? DMAT : DMIS;
        else
                mis += _day[*px-'A'][*py-'A'];

        /* update penalty for del in x seq;
         * favor new del over ongong del
         * ignore MAXGAP if weighting endgaps
         */
        if (endgaps || ndely[yy] < MAXGAP) {
                if (col0[yy] - ins0 >= dely[yy]) {
                        dely[yy] = col0[yy] - (ins0+ins1);
                        ndely[yy] = 1;
                } else {
                        dely[yy] -= ins1;
                        ndely[yy]++;
                }
        } else {
                if (col0[yy] - (ins0+ins1) >= dely[yy]) {
                        dely[yy] = col0[yy] - (ins0+ins1);
                        ndely[yy] = 1;
                } else
                        ndely[yy]++;
        }

        /* update penalty for del in y seq;
         * favor new del over ongong del
         */
        if (endgaps || ndelx < MAXGAP) {
                if (col1[yy-1] - ins0 >= delx) {
                        delx = col1[yy-1] - (ins0+ins1);
                        ndelx = 1;
                } else {
                        delx -= ins1;
                        ndelx++;
                }
        } else {
                if (col1[yy-1] - (ins0+ins1) >= delx) {
                        delx = col1[yy-1] - (ins0+ins1);
                        ndelx = 1;
                } else
                        ndelx++;
        }

        /* pick the maximum score; we're favoring
         * mis over any del and delx over dely
         */
```

FIGURE 4F

```
        id = xx - yy + len1 - 1;
        if (mis >= delx && mis >= dely[yy])
                col1[yy] = mis;
        else if (delx >= dely[yy]) {
                col1[yy] = delx;
                ij = dx[id].ijmp;
                if (dx[id].jp.n[0] && (!dna || (ndelx >= MAXJMP
                && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
                        dx[id].ijmp++;
                        if (++ij >= MAXJMP) {
                                writejmps(id);
                                ij = dx[id].ijmp = 0;
                                dx[id].offset = offset;
                                offset += sizeof(struct jmp) + sizeof(offset);
                        }
                }
                dx[id].jp.n[ij] = ndelx;
                dx[id].jp.x[ij] = xx;
                dx[id].score = delx;
        }
        else {
                col1[yy] = dely[yy];
                ij = dx[id].ijmp;

if (dx[id].jp.n[0] && (!dna || (ndely[yy] >= MAXJMP
                && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
                        dx[id].ijmp++;
                        if (++ij >= MAXJMP) {
                                writejmps(id);
                                ij = dx[id].ijmp = 0;
                                dx[id].offset = offset;
                                offset += sizeof(struct jmp) + sizeof(offset);
                        }
                }
                dx[id].jp.n[ij] = -ndely[yy];
                dx[id].jp.x[ij] = xx;
                dx[id].score = dely[yy];
        }
        if (xx == len0 && yy < len1) {
                /* last col
                 */
                if (endgaps)
                        col1[yy] -= ins0+ins1*(len1-yy);
                if (col1[yy] > smax) {
                        smax = col1[yy];
                        dmax = id;
                }
        }
    }
    if (endgaps && xx < len0)
            col1[yy-1] -= ins0+ins1*(len0-xx);
    if (col1[yy-1] > smax) {
            smax = col1[yy-1];
            dmax = id;
    }
    tmp = col0; col0 = col1; col1 = tmp;
}
(void) free((char *)ndely);
(void) free((char *)dely);
(void) free((char *)col0);(void) free((char *)col1);}
```

FIGURE 4G

```
/*
 *
 * print() -- only routine visible outside this module
 *
 * static:
 * getmat() -- trace back best path, count matches: print()
 * pr_align() -- print alignment of described in array p[]: print()
 * dumpblock() -- dump a block of lines with numbers, stars: pr_align()
 * nums() -- put out a number line: dumpblock()
 * putline() -- put out a line (name, [num], seq, [num]): dumpblock()
 * stars() - -put a line of stars: dumpblock()
 * stripname() -- strip any path and prefix from a seqname
 */

#include "nw.h"

#define SPC        3
#define P_LINE     256        /* maximum output line */
#define P_SPC      3          /* space between name or num and seq */

extern     _day[26][26];
int        olen;              /* set output line length */
FILE       *fx;               /* output file */

print()
{
           int        lx, ly, firstgap, lastgap;       /* overlap */

           if ((fx = fopen(ofile, "w")) == 0) {
                      fprintf(stderr,"%s: can't write %s\n", prog, ofile);
                      cleanup(1);
           }
           fprintf(fx, "<first sequence: %s (length = %d)\n", namex[0], len0);
           fprintf(fx, "<second sequence: %s (length = %d)\n", namex[1], len1);
           olen = 60;
           lx = len0;
           ly = len1;
           firstgap = lastgap = 0;
           if (dmax < len1 - 1) {        /* leading gap in x */
                      pp[0].spc = firstgap = len1 - dmax - 1;
                      ly -= pp[0].spc;
           }
           else if (dmax > len1 - 1) {   /* leading gap in y */
                      pp[1].spc = firstgap = dmax - (len1 - 1);
                      lx -= pp[1].spc;
           }
           if (dmax0 < len0 - 1) {       /* trailing gap in x */
                      lastgap = len0 - dmax0 -1;
                      lx -= lastgap;
           }
           else if (dmax0 > len0 - 1) { /* trailing gap in y */
                      lastgap = dmax0 - (len0 - 1);
                      ly -= lastgap;
           }
           getmat(lx, ly, firstgap, lastgap);
           pr_align();
}
```

print

FIGURE 4H

getmat

```
/*
 * trace back the best path, count matches
 */
static
getmat(lx, ly, firstgap, lastgap)
        int         lx, ly;                     /* "core" (minus endgaps) */
        int         firstgap, lastgap;          /* leading trailing overlap */
{
        int                 nm, i0, i1, siz0, siz1;
        char                outx[32];
        double              pct;
        register            n0, n1;
        register char       *p0, *p1;

        /* get total matches, score
         */
        i0 = i1 = siz0 = siz1 = 0;
        p0 = seqx[0] + pp[1].spc;
        p1 = seqx[1] + pp[0].spc;
        n0 = pp[1].spc + 1;
        n1 = pp[0].spc + 1;

        nm = 0;
        while ( *p0 && *p1 ) {
                if (siz0) {
                        p1++;
                        n1++;
                        siz0--;
                }
                else if (siz1) {
                        p0++;
                        n0++;
                        siz1--;
                }
                else {
                        if (xbm[*p0-'A']&xbm[*p1-'A'])
                                nm++;
                        if (n0++ == pp[0].x[i0])
                                siz0 = pp[0].n[i0++];
                        if (n1++ == pp[1].x[i1])
                                siz1 = pp[1].n[i1++];
                        p0++;
                        p1++;
                }
        }

        /* pct homology:
         * if penalizing endgaps, base is the shorter seq
         * else, knock off overhangs and take shorter core
         */
        if (endgaps)
                lx = (len0 < len1)? len0 : len1;
        else
                lx = (lx < ly)? lx : ly;
        pct = 100.*(double)nm/(double)lx;
        fprintf(fx, "\n");
        fprintf(fx, "< %d match%s in an overlap of %d: %.2f percent similarity\n",
                nm, (nm == 1)? "" : "es", lx, pct);
```

FIGURE 4I

...getmat

```
          fprintf(fx, "<gaps in first sequence: %d", gapx);
          if (gapx) {
                    (void) sprintf(outx, " (%d %s%s)",
                              ngapx, (dna)? "base":"residue", (ngapx == 1)? "":"s");
                    fprintf(fx,"%s", outx);

          fprintf(fx, ", gaps in second sequence: %d", gapy);
          if (gapy) {
                    (void) sprintf(outx, " (%d %s%s)",
                              ngapy, (dna)? "base":"residue", (ngapy == 1)? "":"s");
                    fprintf(fx,"%s", outx);
          }
          if (dna)
                    fprintf(fx,
                    "\n<score: %d (match = %d, mismatch = %d, gap penalty = %d + %d per base)\n",
                    smax, DMAT, DMIS, DINS0, DINS1);
          else
                    fprintf(fx,
                    "\n<score: %d (Dayhoff PAM 250 matrix, gap penalty = %d + %d per residue)\n",
                    smax, PINS0, PINS1);
          if (endgaps)
                    fprintf(fx,
                    "<endgaps penalized. left endgap: %d %s%s, right endgap: %d %s%s\n",
                    firstgap, (dna)? "base" : "residue", (firstgap == 1)? "" : "s",
                    lastgap, (dna)? "base" : "residue", (lastgap == 1)? "" : "s");
          else
                    fprintf(fx, "<endgaps not penalized\n");
}

static              nm;                 /* matches in core -- for checking */
static              lmax;               /* lengths of stripped file names */
static              ij[2];              /* jmp index for a path */
static              nc[2];              /* number at start of current line */
static              ni[2];              /* current elem number -- for gapping */
static              siz[2];
static char         *ps[2];             /* ptr to current element */
static char         *po[2];             /* ptr to next output char slot */
static char         out[2][P_LINE];     /* output line */
static char         star[P_LINE];       /* set by stars() */

/*
 * print alignment of described in struct path pp[]
 */
static
pr_align()
{
          int                 nn;       /* char count */
          int                 more;
          register            i;

          for (i = 0, lmax = 0; i < 2; i++) {
                    nn = stripname(namex[i]);
                    if (nn > lmax)
                              lmax = nn;

                    nc[i] = 1;
                    ni[i] = 1;
                    siz[i] = ij[i] = 0;
                    ps[i] = seqx[i];
                    po[i] = out[i];
```

pr_align

FIGURE 4J

...pr_align

```
        for (nn = nm = 0, more = 1; more; ) {
                for (i = more = 0; i < 2; i++) {
                        /*
                         * do we have more of this sequence?
                         */
                        if (!*ps[i])
                                continue;

                        more++;

                        if (pp[i].spc) {        /* leading space */
                                *po[i]++ = ' ';
                                pp[i].spc--;
                        }
                        else if (siz[i]) {      /* in a gap */
                                *po[i]++ = '-';
                                siz[i]--;
                        }
                        else {                  /* we're putting a seq element
                                                 */
                                *po[i] = *ps[i];
                                if (islower(*ps[i]))
                                        *ps[i] = toupper(*ps[i]);
                                po[i]++;
                                ps[i]++;

                                /*
                                 * are we at next gap for this seq?
                                 */
                                if (ni[i] == pp[i].x[ij[i]]) {
                                        /*
                                         * we need to merge all gaps
                                         * at this location
                                         */
                                        siz[i] = pp[i].n[ij[i]++];
                                        while (ni[i] == pp[i].x[ij[i]])
                                                siz[i] += pp[i].n[ij[i]++];
                                }
                                ni[i]++;
                        }
                }
                if (++nn == olen || !more && nn) {
                        dumpblock();
                        for (i = 0; i < 2; i++)
                                po[i] = out[i];
                        nn = 0;
                }
        }
}

/*
 * dump a block of lines, including numbers, stars: pr_align()
 */
static
dumpblock()
{
        register  i;

        for (i = 0; i < 2; i++)
                *po[i]-- = '\0';
```

dumpblock

FIGURE 4K

...dumpblock

```
        (void) putc('\n', fx);
        for (i = 0; i < 2; i++) {
                if (*out[i] && (*out[i] != ' ' || *(po[i]) != ' ')) {
                        if (i == 0)
                                nums(i);
                        if (i == 0 && *out[1])
                                stars();
                        putline(i);
                        if (i == 0 && *out[1])
                                fprintf(fx, star);
                        if (i == 1)
                                nums(i);
                }
        }
}

/*
 * put out a number line: dumpblock()
 */
static
```

nums

```
nums(ix)
        int     ix;     /* index in out[] holding seq line */
{
        char            nline[P_LINE];
        register        i, j;
        register char   *pn, *px, *py;

        for (pn = nline, i = 0; i < lmax+P_SPC; i++, pn++)
                *pn = ' ';
        for (i = nc[ix], py = out[ix]; *py; py++, pn++) {
                if (*py == ' ' || *py == '-')
                        *pn = ' ';
                else {
                        if (i%10 == 0 || (i == 1 && nc[ix] != 1)) {
                                j = (i < 0)? -i : i;
                                for (px = pn; j; j /= 10, px--)
                                        *px = j%10 + '0';
                                if (i < 0)
                                        *px = '-';
                        }
                        else
                                *pn = ' ';
                        i++;
                }
        }
        *pn = '\0';
        nc[ix] = i;
        for (pn = nline; *pn; pn++)
                (void) putc(*pn, fx);
        (void) putc('\n', fx);
}

/*
 * put out a line (name, [num], seq, [num]): dumpblock()
 */
static
```

putline

```
putline(ix)
        int     ix;
{
```

FIGURE 4L

...putline

```
        int                     i;
        register char           *px;

        for (px = namex[ix], i = 0; *px && *px != ':'; px++, i++)
                (void) putc(*px, fx);
        for (; i < lmax+P_SPC; i++)
                (void) putc(' ', fx);

        /* these count from 1:
         * ni[] is current element (from 1)
         * nc[] is number at start of current line
         */
        for (px = out[ix]; *px; px++)
                (void) putc(*px&0x7F, fx);
        (void) putc('\n', fx);
}
```

stars

```
/*
 * put a line of stars (seqs always in out[0], out[1]): dumpblock()
 */
static
stars()
{
        int                     i;
        register char           *p0, *p1, cx, *px;

        if (!*out[0] || (*out[0] == ' ' && *(po[0]) == ' ') ||
           !*out[1] || (*out[1] == ' ' && *(po[1]) == ' '))
                return;
        px = star;
        for (i = lmax+P_SPC; i; i--)
                *px++ = ' ';

        for (p0 = out[0], p1 = out[1]; *p0 && *p1; p0++, p1++) {
                if (isalpha(*p0) && isalpha(*p1)) {

                        if (xbm[*p0-'A']&xbm[*p1-'A']) {
                                cx = '*';
                                nm++;
                        }
                        else if (!dna && _day[*p0-'A'][*p1-'A'] > 0)
                                cx = '.';
                        else
                                cx = ' ';
                }
                else
                        cx = ' ';
                *px++ = cx;
        }
        *px++ = '\n';
        *px = '\0';
}
```

FIGURE 4M

stripname

```
/*
 * strip path or prefix from pn, return len: pr_align()
 */
static
stripname(pn)
          char      *pn;      /* file name (may be path) */
{
          register char       *px, *py;

          py = 0;
          for (px = pn; *px; px++)
                    if (*px == '/')
                              py = px + 1;
          if (py)
                    (void) strcpy(pn, py);
          return(strlen(pn));

}
```

FIGURE 4N

```
/*
 * cleanup() -- cleanup any tmp file
 * getseq() -- read in seq, set dna, len, maxlen
 * g_calloc() -- calloc() with error checkin
 * readjmps() -- get the good jmps, from tmp file if necessary
 * writejmps() -- write a filled array of jmps to a tmp file: nw()
 */
#include "nw.h"
#include <sys/file.h>

char    *jname = "/tmp/homgXXXXXX";                /* tmp file for jmps */
FILE    *fj;

int     cleanup();                                 /* cleanup tmp file */
long    lseek();

/*
 * remove any tmp file if we blow
 */
cleanup(i)                                                                    cleanup
        int     i;
{
        if (fj)
                (void) unlink(jname);
        exit(i);
}

/*
 * read, return ptr to seq, set dna, len, maxlen
 * skip lines starting with ';', '<', or '>'
 * seq in upper or lower case
 */
char    *
getseq(file, len)                                                             getseq
        char    *file;  /* file name */
        int     *len;   /* seq len */
{
        char            line[1024], *pseq;
        register char   *px, *py;
        int             natgc, tlen;
        FILE            *fp;

        if ((fp = fopen(file,"r")) == 0) {
                fprintf(stderr,"%s: can't read %s\n", prog, file);
                exit(1);
        }
        tlen = natgc = 0;
        while (fgets(line, 1024, fp)) {
                if (*line == ';' || *line == '<' || *line == '>')
                        continue;
                for (px = line; *px != '\n'; px++)
                        if (isupper(*px) || islower(*px))
                                tlen++;
        }
        if ((pseq = malloc((unsigned)(tlen+6))) == 0) {
                fprintf(stderr,"%s: malloc() failed to get %d bytes for %s\n", prog, tlen+6, file);
                exit(1);
        }
        pseq[0] = pseq[1] = pseq[2] = pseq[3] = '\0';
```

FIGURE 40

```
                                                                                  ...getseq
          py = pseq + 4;
          *len = tlen;
          rewind(fp);

          while (fgets(line, 1024, fp)) {
                    if (*line == ';' || *line == '<' || *line == '>')
                              continue;
                    for (px = line; *px != '\n'; px++) {
                              if (isupper(*px))
                                        *py++ = *px;
                              else if (islower(*px))
                                        *py++ = toupper(*px);
                              if (index("ATGCU",*(py-1)))
                                        natgc++;
                    }
          }
          *py++ = '\0';
          *py = '\0';
          (void) fclose(fp);
          dna = natgc > (tlen/3);
          return(pseq+4);
}

char      *
g_calloc(msg, nx, sz)                                                             g_calloc
          char      *msg;               /* program, calling routine */
          int       nx, sz;             /* number and size of elements */
{
          char                *px, *calloc();

          if ((px = calloc((unsigned)nx, (unsigned)sz)) == 0) {
                    if (*msg) {
                              fprintf(stderr, "%s: g_calloc() failed %s (n=%d, sz=%d)\n", prog, msg, nx, sz);
                              exit(1);
                    }
          }
          return(px);
}

/*
 * get final jmps from dx[] or tmp file, set pp[], reset dmax: main()
 */
readjmps()                                                                        readjmps
{
          int                 fd = -1;
          int                 siz, i0, i1;
          register  i, j, xx;

          if (fj) {
                    (void) fclose(fj);
                    if ((fd = open(jname, O_RDONLY, 0)) < 0) {
                              fprintf(stderr, "%s: can't open() %s\n", prog, jname);
                              cleanup(1);
                    }
          }
          for (i = i0 = i1 = 0, dmax0 = dmax, xx = len0; ; i++) {
                    while (1) {
                              for (j = dx[dmax].ijmp; j >= 0 && dx[dmax].jp.x[j] >= xx; j--)
                                        ;
```

FIGURE 4P

...readjmps

```
                        if (j < 0 && dx[dmax].offset && fj) {
                                (void) lseek(fd, dx[dmax].offset, 0);
                                (void) read(fd, (char *)&dx[dmax].jp, sizeof(struct jmp));
                                (void) read(fd, (char *)&dx[dmax].offset, sizeof(dx[dmax].offset));
                                dx[dmax].ijmp = MAXJMP-1;
                        }
                        else
                                break;
                }
                if (i >= JMPS) {
                        fprintf(stderr, "%s: too many gaps in alignment\n", prog);
                        cleanup(1);
                }
                if (j >= 0) {
                        siz = dx[dmax].jp.n[j];
                        xx = dx[dmax].jp.x[j];
                        dmax += siz;
                        if (siz < 0) {                  /* gap in second seq */
                                pp[1].n[i1] = -siz;
                                xx += siz;

                                /* id = xx - yy + len1 - 1
                                 */
                                pp[1].x[i1] = xx - dmax + len1 - 1;
                                gapy++;
                                ngapy -= siz;
/* ignore MAXGAP when doing endgaps */
                                siz = (-siz < MAXGAP || endgaps)? -siz : MAXGAP;
                                i1++;
                        }
                        else if (siz > 0) {  /* gap in first seq */
                                pp[0].n[i0] = siz;
                                pp[0].x[i0] = xx;
                                gapx++;
                                ngapx += siz;
/* ignore MAXGAP when doing endgaps */
                                siz = (siz < MAXGAP || endgaps)? siz : MAXGAP;
                                i0++;
                        }
                }
                else
                        break;
        }

        /* reverse the order of jmps
         */
        for (j = 0, i0--; j < i0; j++, i0--) {
                i = pp[0].n[j]; pp[0].n[j] = pp[0].n[i0]; pp[0].n[i0] = i;
                i = pp[0].x[j]; pp[0].x[j] = pp[0].x[i0]; pp[0].x[i0] = i;
        }
        for (j = 0, i1--; j < i1; j++, i1--) {
                i = pp[1].n[j]; pp[1].n[j] = pp[1].n[i1]; pp[1].n[i1] = i;
                i = pp[1].x[j]; pp[1].x[j] = pp[1].x[i1]; pp[1].x[i1] = i;
        }
        if (fd >= 0)
                (void) close(fd);
        if (fj) {
                (void) unlink(jname);
                fj = 0;
                offset = 0;}}
```

FIGURE 4Q

writejmps

```
/*
 * write a filled jmp struct offset of the prev one (if any): nw()
 */
writejmps(ix)
	int	ix;
{
	char	*mktemp();

	if (!fj) {
		if (mktemp(jname) < 0) {
			fprintf(stderr, "%s: can't mktemp() %s\n", prog, jname);
			cleanup(1);
		}
		if ((fj = fopen(jname, "w")) == = 0) {
			fprintf(stderr, "%s: can't write %s\n", prog, jname);
			exit(1);
		}
	}
	(void) fwrite((char *)&dx[ix].jp, sizeof(struct jmp), 1, fj);
	(void) fwrite((char *)&dx[ix].offset, sizeof(dx[ix].offset), 1, fj);
}
```

FIGURE 5

```
GTGCTCTCCGAGGACAAGCAGGAGGNGGTGGAGCTGGTGAAGCACCATCTGTGGGCTCTG
GAAGTGTGCTACATCTCAGCCTTGGTCTTGTCCTGCTTACTCACCTTCCTGGTCCTGATG
CGCTCACTGGTGACACACAGGACCAACCTTCGAGCTCTGCACCGAGGAGCTGCCCTGGAC
TTGAGTCCCTTGCATCGGAGTCCCCATCCCTCCCGCCAAGCCATATTCTGTTGGATGAGC
TTCAGTGCCTACCAGACAGCCTTTATCTGCCTTGGGCTCCTGGTGCAGCAGATCATCTTC
TTCCTGGGAACCACGGCCCTGGCCTTCCTGGTGCTCATGCCTGTGCTCCATGGCAGGAAC
CTCCTGCTCTTCCGTTCCCTGGAGTCCTCGTGGCCCTTCTGGCTGACTTTGGCCCTGGCT
GTGATCCTGCAGAACATGGCAGCCCATTGGGTCTTCCTGGAGACTCATGATGGACACCCA
CAGCTGACCAACCGGCGAGTGCTCTATGCAGCCACCTTTCTTCTCTTCCCCCTCAATGTG
CTGGTGGGTGCCATGGTGGCCACCTGGCGAGTGCTCCTCTCTGCCCTCTACAACGCCATC
CACCTTGGCCAGATGGACCTCAGCCTGCTGCCACCGAGAGCCGCCACTCTCGACCCCGGC
TACTACACGTACCGAA
```

FIGURE 6

```
CACAACCAGCCACCCCTCTAGGATCCCAGCCCAGCTGGTGCTGGGCTCAGAGGAGAAGGC
CCCGTGTTGGGAGCACCCTGCTTGCCTGGAGGGACAAGTTTCCGGGAGAGATCAATAAAG
GAAAGGAAAGAGACAAGGAAGGGAGAGGTCAGGAGAGCGCTTGATTGGAGGAGAAGGGCC
AGAGAATGTCGTCCCAGCCAGCAGGGAACCAGACCTCCCCCGGGGCCACAGAGGACTACT
CCTATGGCAGCTGGTACATCGATGAGCCCCAGGGGGGCGAGGAGCTCCAGCCAGAGGGGG
AAGTGCCCTCCTGCCACACCAGCATACCACCCGGCCTGTACCACGCCTGCCTGGCCTCGC
TGTCAATCCTTGTGCTGCTGCTCCTGGCCATGCTGGTGAGGCGCCGCCAGCTCTGGCCTG
ACTGTGTGCGTGGCAGGCCCGGCCTGCCCAGGCCCCGGGCAGTGCCTGCTGCTGTTTTCA
TGGTCCTCCTGAGCTCCCTGTGTTTGCTGCTCCCCGACGAGGACGCATTGCCCTTCCTGA
CTCTCGCCTCAGCACCCAGCCAAGATGGGAAAACTGAGGCTCCAAGAGGGGCCTGGAAGA
TACTGGGACTGTTCTATTATGCTGCCCTCTACTACCCTCTGGCTGCCTGTGCCACGGCTG
GCCACACAGCTGCACACCTGCTCGGCAGCACGCTGTCCTGGGCCCACCTTGGGGTCCAGG
TCTGGCAGAGGGCAGAGTGTCCCCAGGTGCCCAAGATCTACAAGTACTACTCCCTGCTGG
CCTCCCTGCCTCTCCTGCTGGGCCTCGGATTCCTGAGCCTTTGGTACCCTGTGCAGCTGG
TGAGAAGCTTCAGCCGTAGGACAGGAGCAGGCTCCAAGGGGCTGCAGAGCAGCTACTCTG
AGGAATATCTGAGGAACCTCCTTTGCAGGAAGAAGCTGGGAAGCAGCTACCACACCTCCA
AGCATGGCTTCCTGTCCTGGGCCCGCGTCTGCTTGAGACACTGCATCTACACTCCACAGC
CAGGATTCCATCTCCCGCTGAAGCTGGTGCTTTCAGCTACACTGACAGGGACGGCCATTT
ACCAGGTGGCCCTGCTGCTGCTGGTGGGCGTGGTACCCACTATCCAGAAGGTGAGGGCAG
GGGTCACCACGGATGTCTCCTACCTGCTGGCCGGCTTTGGAATCGTGCTCTCCGAGGACA
AGCAGGAGGTGGTGGAGCTGGTGAAGCACCATCTGTGGGCTCTGGAAGTGTGCTACATCT
CAGCCTTGGTCTTGTCCTGCTTACTCACCTTCCTGGTCCTGATGCGCTCACTGGTGACAC
ACAGGACCAACCTTCGAGCTCTGCACCGAGGAGCTGCCCTGGACTTGAGTCCCTTGCATC
GGAGTCCCCATCCCTCCCGCCAAGCCATATTCTGTTGGATGAGCTTCAGTGCCTACCAGA
CAGCCTTTATCTGCCTTGGGCTCCTGGTGCAGCAGATCATCTTCTTCCTGGGAACCACGG
CCCTGGCCTTCCTGGTGCTCATGCCTGTGCTCCATGGCAGGAACCTCCTGCTCTTCCGTT
CCCTGGAGTCCTCGTGGCCCTTCTGGCTGACTTTGGCCCTGGCTGTGATCCTGCAGAACA
TGGCAGCCCATTGGGTCTTCCTGGAGACTCATGATGGACACCCACAGCTGACCAACCGGC
GAGTGCTCTATGCAGCCACCTTTCTTCTCTTCCCCCTCAATGTGCTGGTGGGTGCCATAG
TGGCCACCTGGCGAGTGCTCCTCTCTGCCCTCTACAACGCCATCCACCTTGGCCAGATGG
ACCTCAGCCTGCTGCCACCGAGAGCCGCCACTCTCGACCCCGGCTACTACACGTACCGAA
ACTTCTTGAAGATTGAAGTCAGCCAGTCGCATCCAGCCATGACAGCCTTCTGCTCCCTGC
TCCTGCAAGCGCAGAGCCTCCTACCCAGGACCATGGCAGCCCCCCAGGACAGCCTCAGAC
CAGGGGAGGAAGACGAAGGGATGCAGCTGCTACAGACAAAGGACTCCATGGCCAAGGGAG
CTAGGCCCGGGGCCAGCCGCGGCAGGGCTCGCTGGGGTCTGGCCTACACGCTGCTGCACA
ACCCAACCCTGCAGGTCTTCCGCAAGACGGCCCTGTTGGGTGCCAATGGTGCCCAGCCCT
GAGGGCAGGGAAGGTCAACCCACCTGCCCATCTGTGCTGAGGCATGTTCCTGCCTACCAC
CTCCTCCCTCCCCGGCTCTCCTCCCAGCATCACACCAGCCATGCAGCCAGCAGGTCCTCC
GGATCACTGTGGTTGGGTGGAGGTCTGTCTGCACTGGGAGCCTCAGGAGGGCTCTGCTCC
ACCCACTTGGCTATGGGAGAGCCAGCAGGGGTTCTGGAGAAAGAAACTGGTGGGTTAGGG
CCTTGGTCCAGGAGCCAGTTGAGCCAGGGCAGCCACATCCAGGCGTCTCCCTACCCTGGC
TCTGCCATCAGCCTTGAAGGGCCTCGATGAAGCCTTCTCTGGAACCACTCCAGCCCAGCT
CCACCTCAGCCTTGGCCTTCACGCTGTGGAAGCAGCCAAGGCACTTCCTCACCCCCTCAG
CGCCACGGACCTCTCTGGGGAGTGGCCGGAAAGCTCCCGGGCCTCTGGCCTGCAGGGCAG
CCCAAGTCATGACTCAGACCAGGTCCCACACTGAGCTGCCCACACTCGAGAGCCAGATAT
TTTTGTAGTTTTTATGCCTTTGGCTATTATGAAAGAGGTTAGTGTGTTCCCTGCAATAAA
CTTGTTCCTGAGAAAAA
```

FIGURE 7

```
MSSQPAGNQTSPGATEDYSYGSWYIDEPQGGEELQPEGEVPSCHTSIPPGLYHACLASL
SILVLLLLAMLVRRRQLWPDCVRGRPGLPRPRAVPAAVFMVLLSSLCLLLPDEDALPFL
TLASAPSQDGKTEAPRGAWKILGLFYYAALYYPLAACATAGHTAAHLLGSTLSWAHLGV
QVWQRAECPQVPKIYKYYSLLASLPLLLGLGFLSLWYPVQLVRSFSRRTGAGSKGLQSS
YSEEYLRNLLCRKKLGSSYHTSKHGFLSWARVCLRHCIYTPQPGFHLPLKLVLSATLTG
TAIYQVALLLLVGVVPTIQKVRAGVTTDVSYLLAGFGIVLSEDKQEVVELVKHHLWALE
VCYISALVLSCLLTFLVLMRSLVTHRTNLRALHRGAALDLSPLHRSPHPSRQAIFCWMS
FSAYQTAFICLGLLVQQIIFFLGTTALAFLVLMPVLHGRNLLLFRSLESSWPFWLTLAL
AVILQNMAAHWVFLETHDGHPQLTNRRVLYAATFLLFPLNVLVGAIVATWRVLLSALYN
AIHLGQMDLSLLPPRAATLDPGYYTYRNFLKIEVSQSHPAMTAFCSLLLQAQSLLPRTM
AAPQDSLRPGEEDEGMQLLQTKDSMAKGARPGASRGRARWGLAYTLLHNPTLQVFRKTA
LLGANGAQP
```

Important features of the protein:

Signal peptide:
none

Transmembrane domain:
54-71
93-111
140-157
197-214
291-312
356-371
425-444
464-481
505-522

Motif name: N-glycosylation site.

8-12

Motif name: N-myristoylation site.

50-56
167-173
232-238
308-314
332-338
516-522
618-624
622-628
631-637
652-658

Motif name: Prokaryotic membrane lipoprotein lipid attachment site.

355-366

Motif name: ATP/GTP-binding site motif A (P-loop).

123-131

FIGURE 8

Stra6 Variant Clones

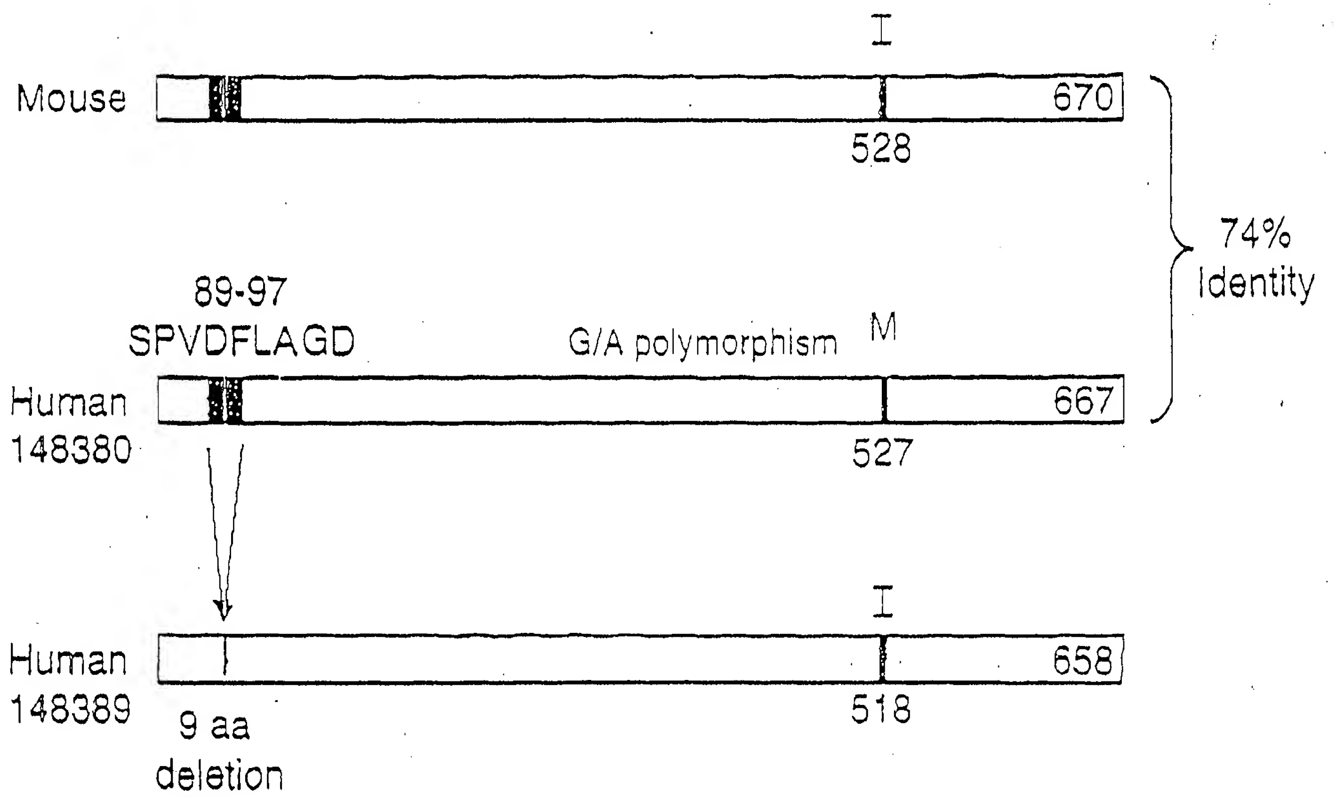
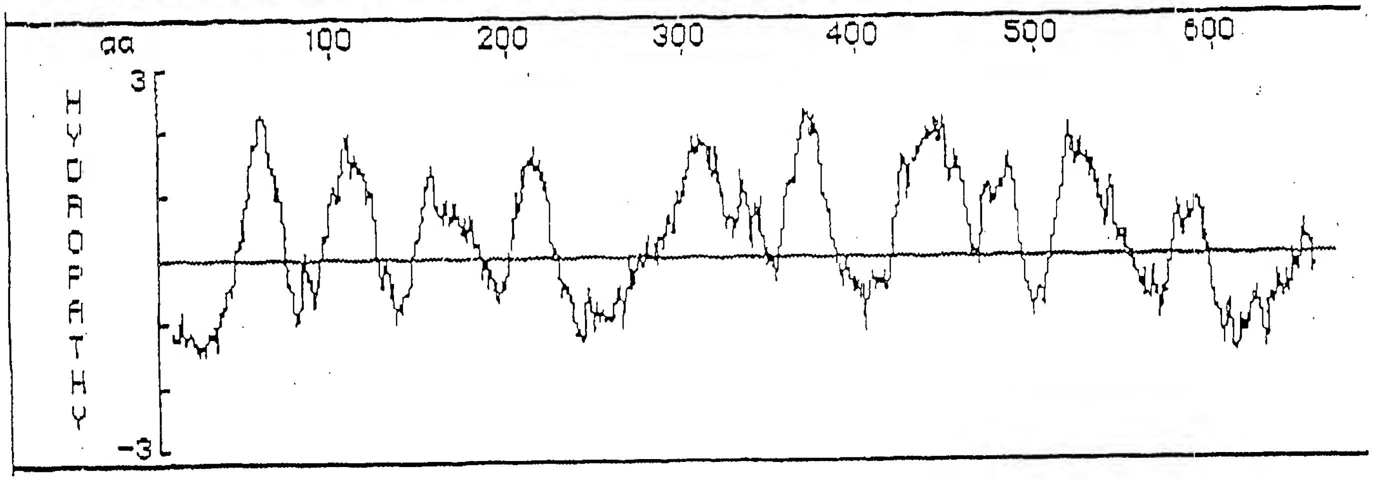


FIGURE 9

Hydrophobicity Plot of Human Stra6



- 3 kb mRNA
- 667 Amino Acids -->50% Residues Hydrophobic
- 73.5 kDa Protein

- 9 Potential Transmembrane Domains

FIGURE 10

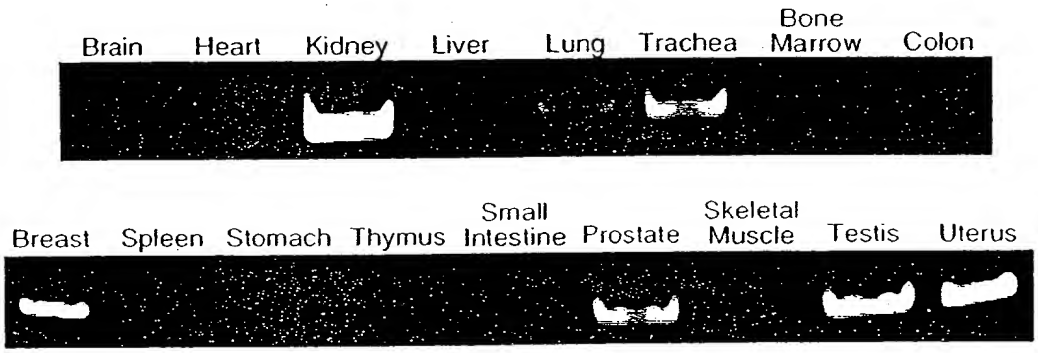

Brain
Heart
Kidney
Liver
Lung
Trachea
Bone Marrow
Colon
Breast
Spleen
Stomach
Thymus
Small Intestine
Prostate
Skeletal Muscle
Testis
Uterus

FIGURE 11

Stra6 RNA Expression in Human Colon Tumor Tissue

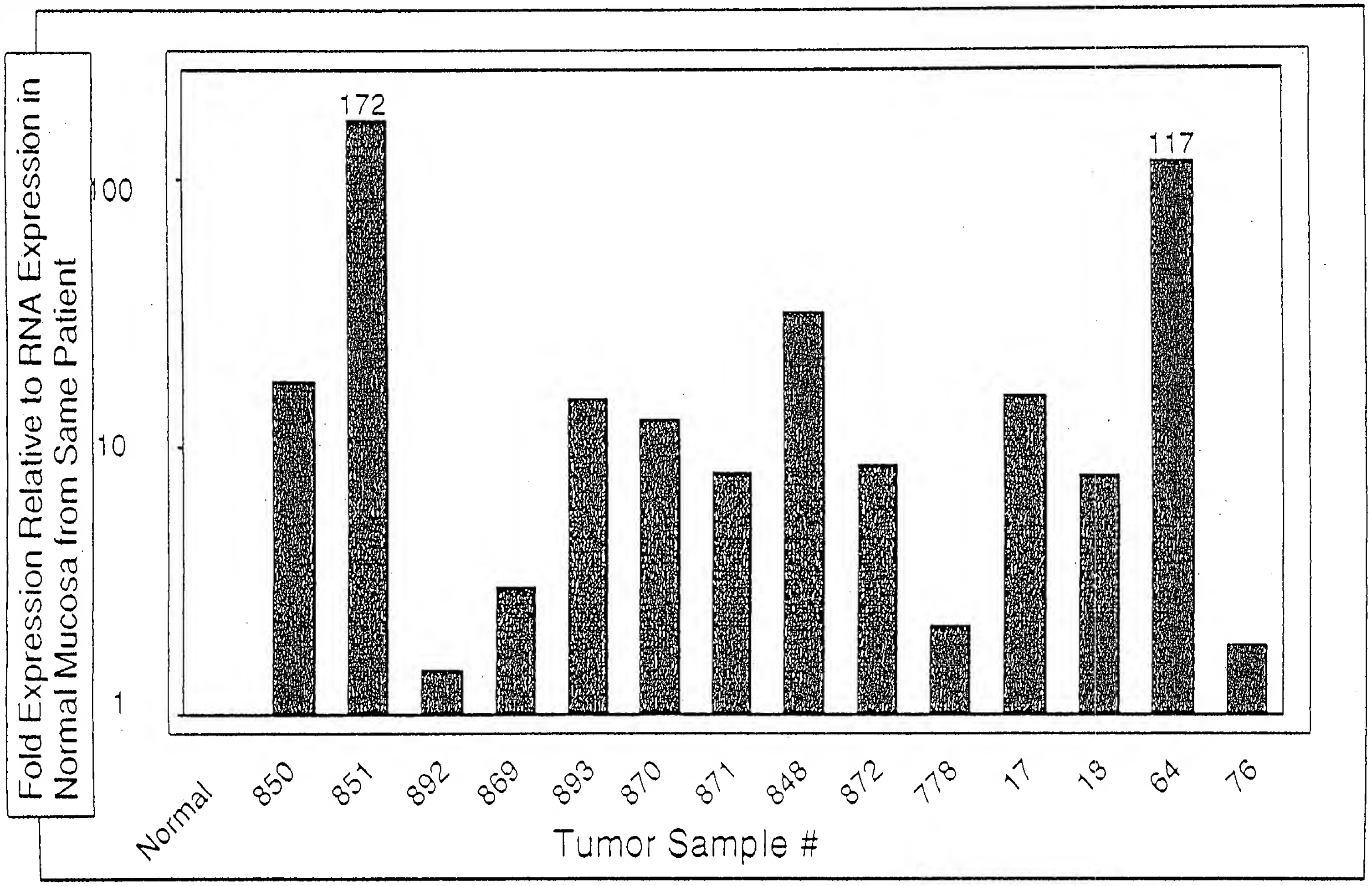
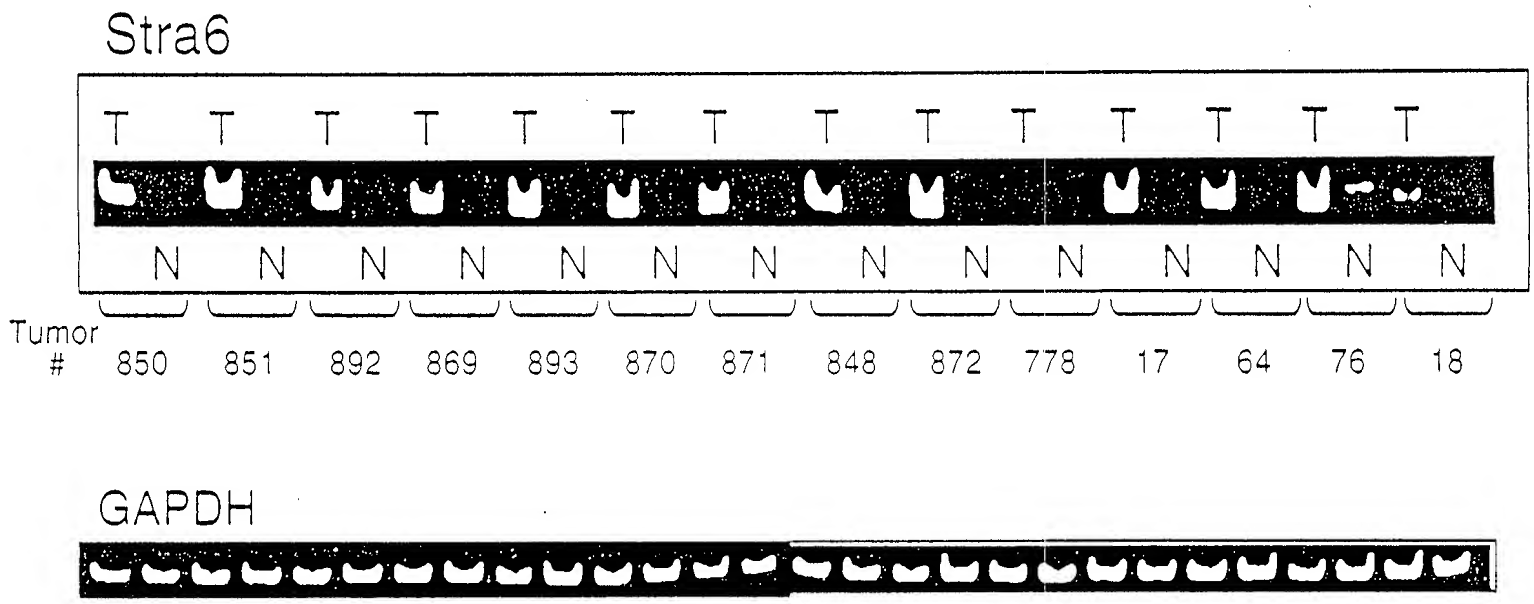


FIGURE 12A

Stra6 RNA Expression in Human Colon Tumor Tissue vs Normal Mucosa From the Same Patient

Taqman Product Analysis After 40 Cycles



WJ TM #84 h.Stra6 Primer Set #4 1/4/00

FIGURE 12B

C

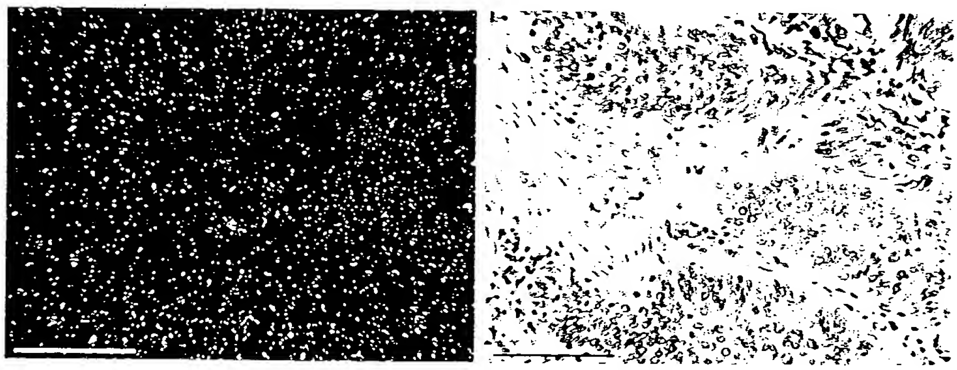


FIGURE 13

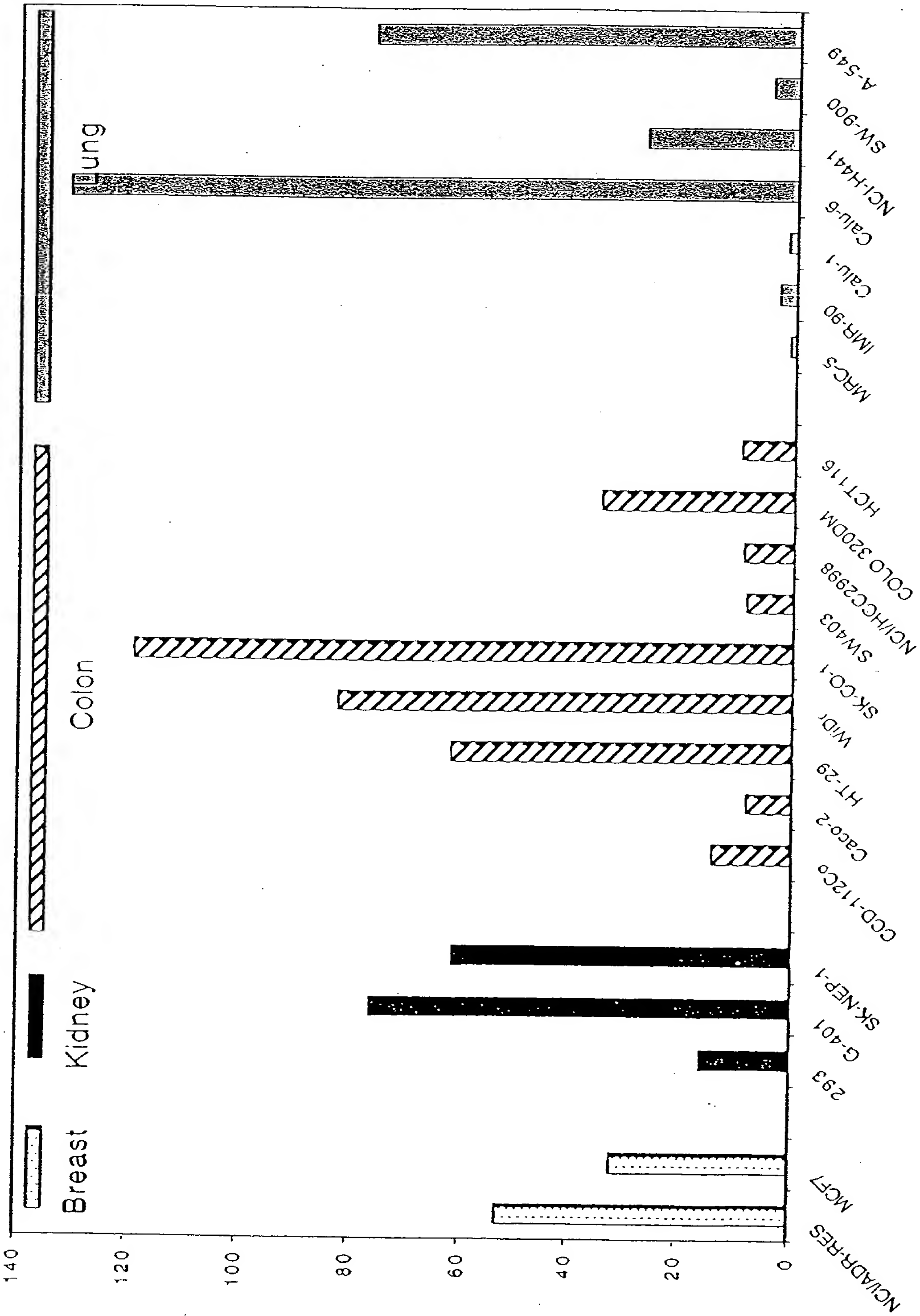
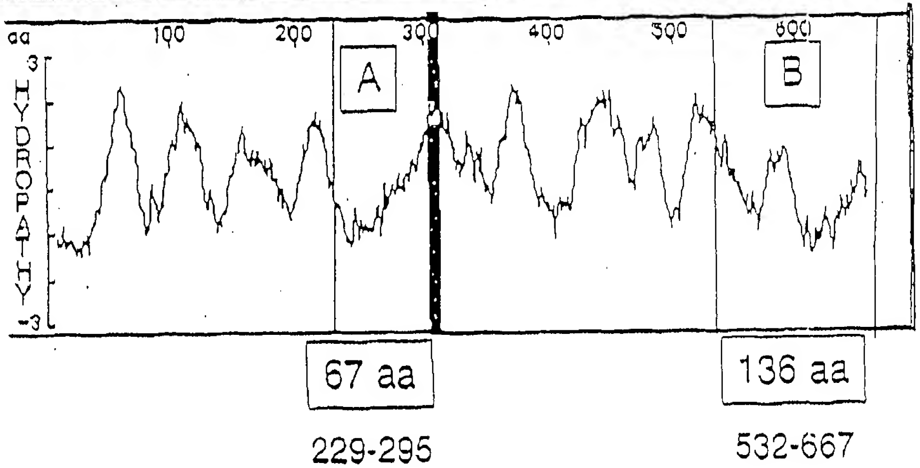
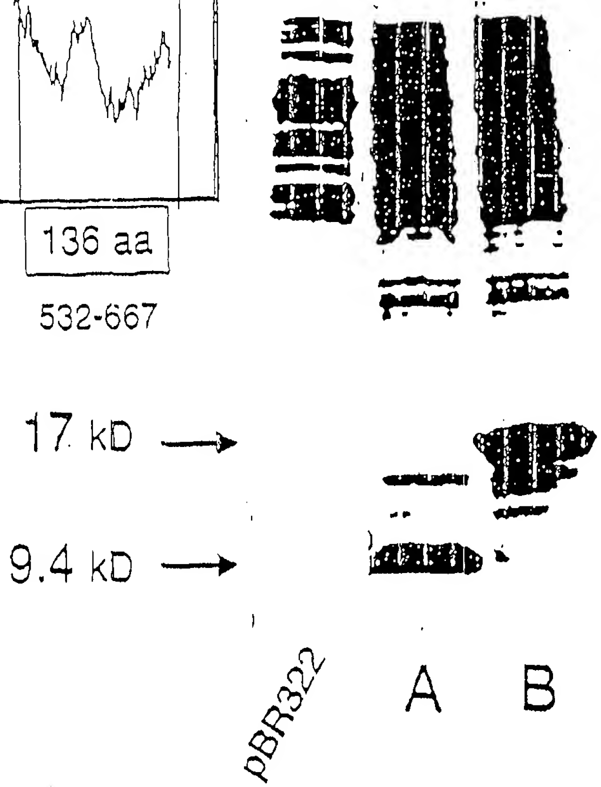

0
20
40
60
80
100
120
140
Breast
Kidney
Colon
Lung
NCI/ADR-RES
MCF7
293
G-401
SK-NEP-1
CCD-112Co
Caco-2
HT-29
WiDr
SK-CO-1
SW403
NCI/HCC2998
COLO 320DM
HCT116
MRC-5
IMR-90
Calu-1
Calu-6
NCI-H441
SW-900
A-549

FIGURE 14

Stra6 Peptide Expression in E. coli

Poly-His Cleavable Leader at N-Terminus





500 ml Culture
15 µl/Lane

Estimate:
~100 µg/ml

~50 mg/500 ml

Dan Yansura

FIGURE 15

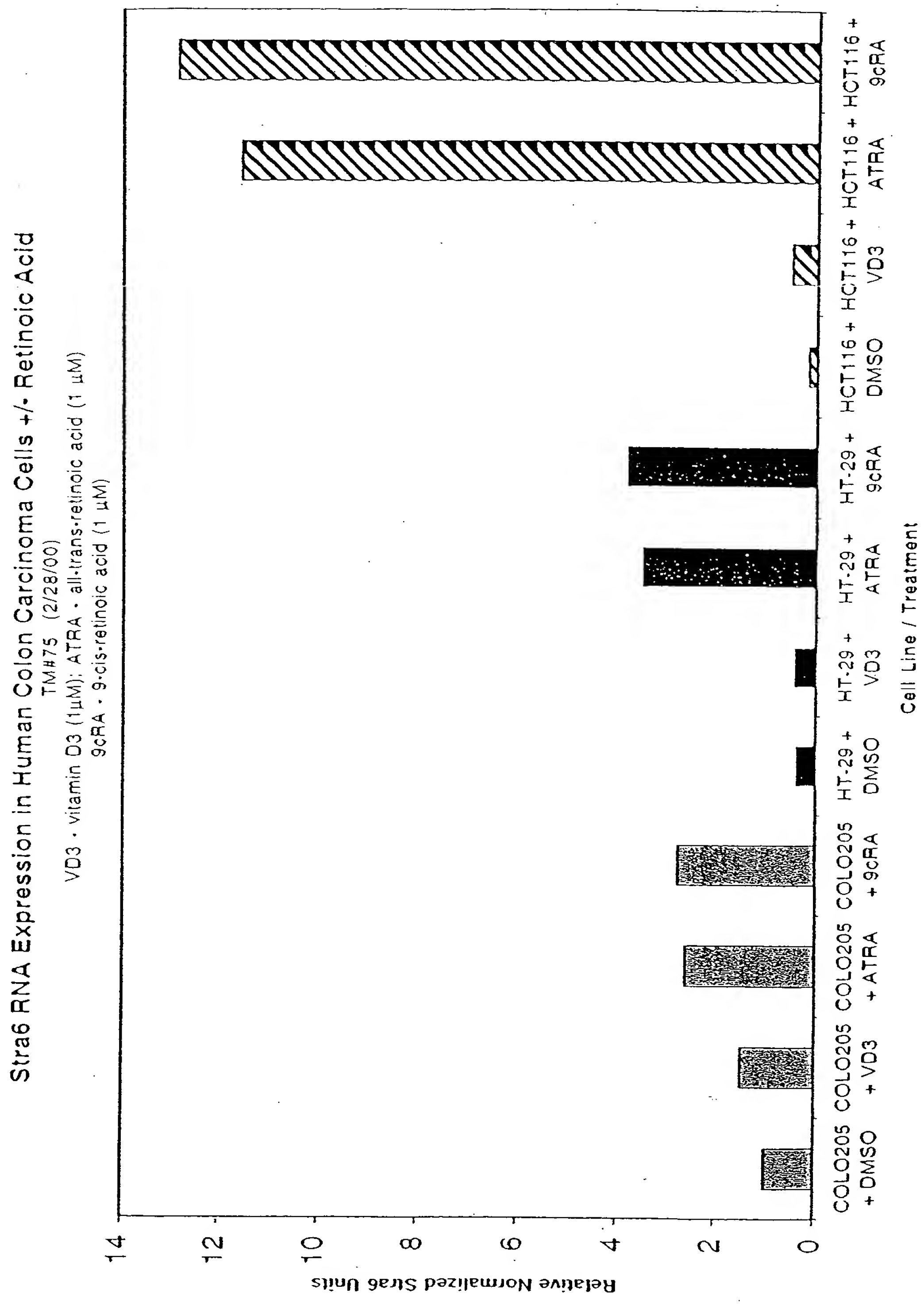

Stra6 RNA Expression in Human Colon Carcinoma Cells +/- Retinoic Acid
TM#75 (2/28/00)
VD3 - vitamin D3 (1μM); ATRA - all-trans-retinoic acid (1 μM)
9cRA - 9-cis-retinoic acid (1 μM)
Relative Normalized Stra6 Units
0
2
4
6
8
10
12
14
COLO205 + DMSO
COLO205 + VD3
COLO205 + ATRA
COLO205 + 9cRA
HT-29 + DMSO
HT-29 + VD3
HT-29 + ATRA
HT-29 + 9cRA
HCT116 + DMSO
HCT116 + VD3
HCT116 + ATRA
HCT116 + 9cRA
Cell Line / Treatment

FIGURE 16

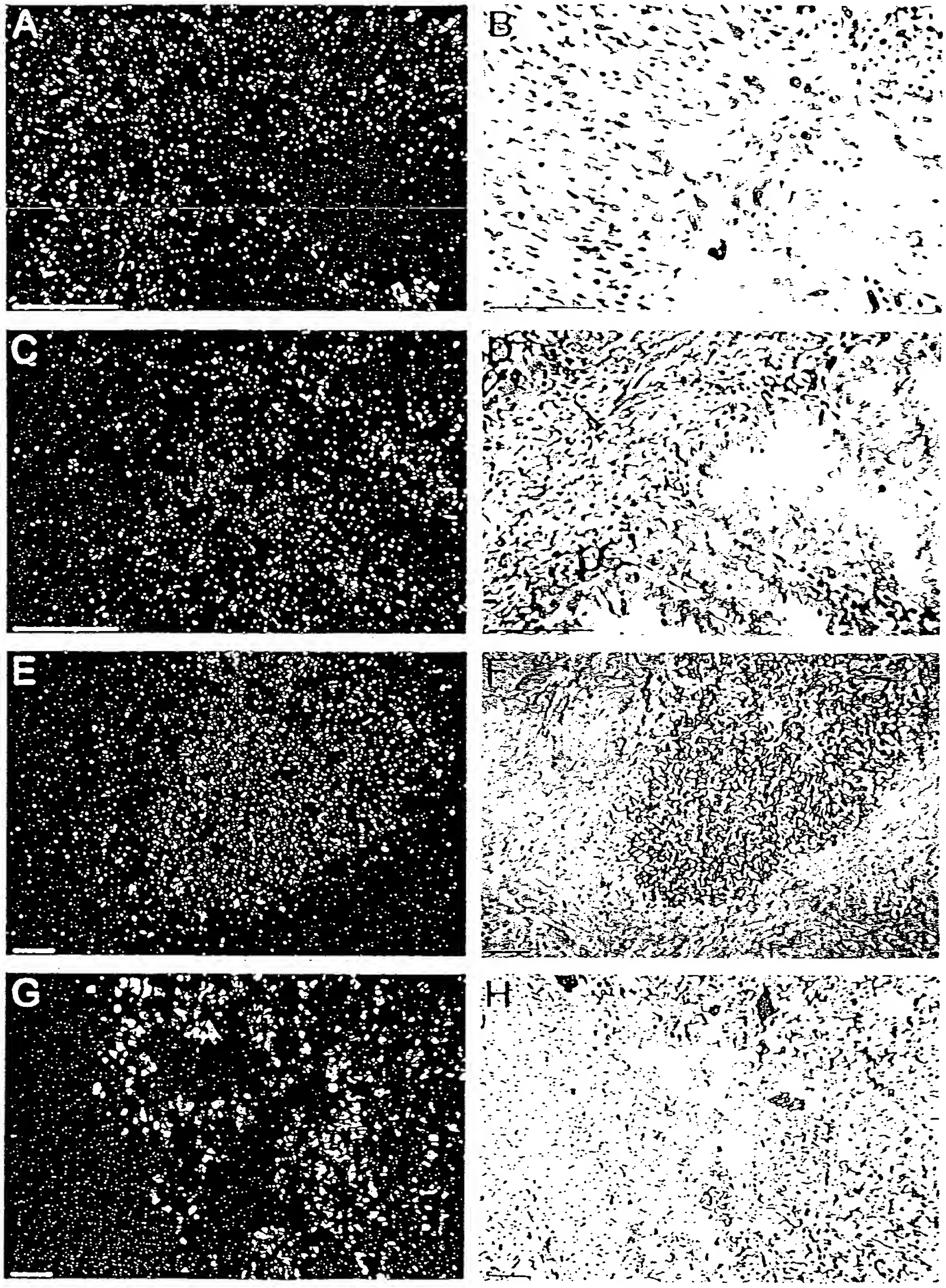
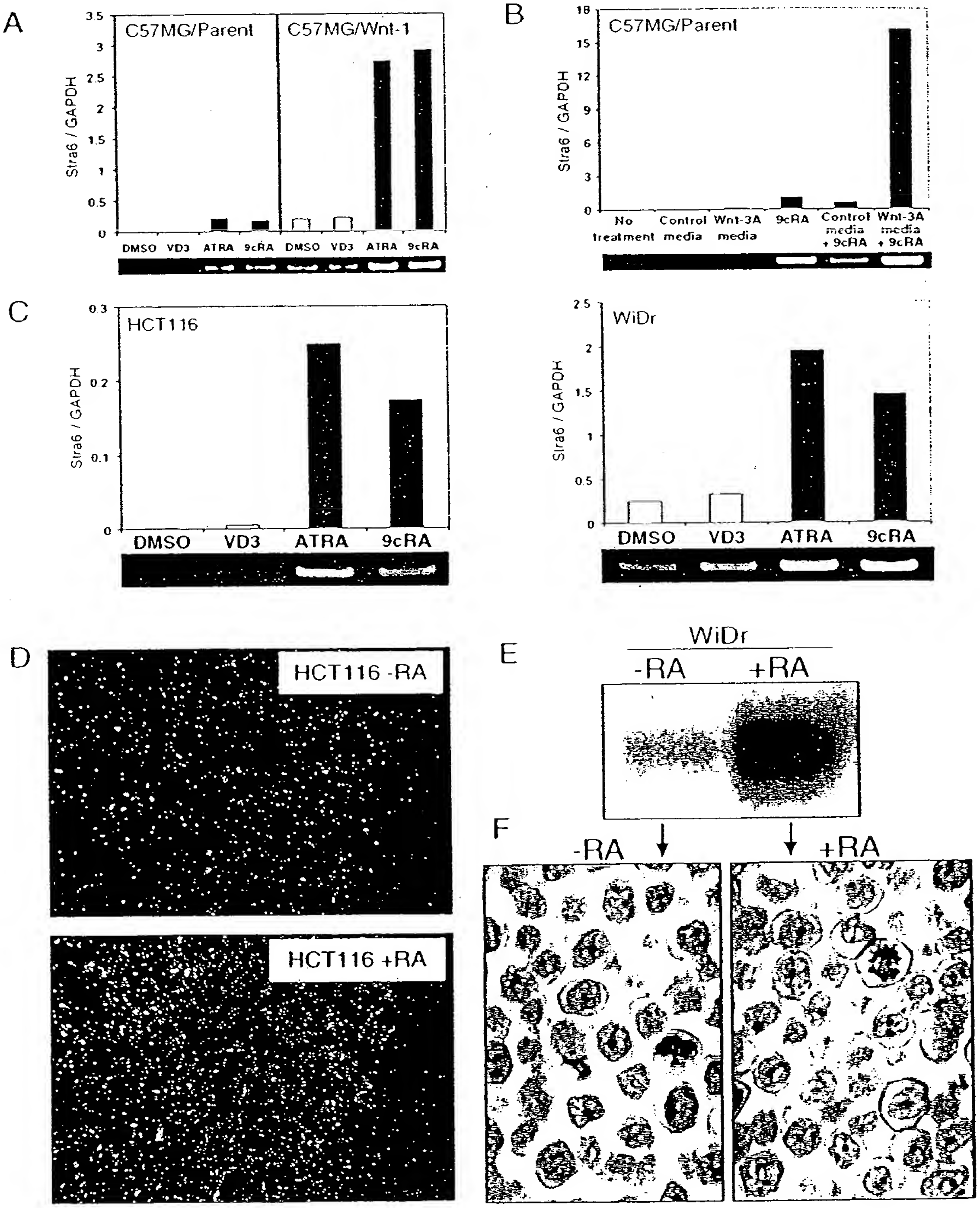


FIGURE 17



A
C57MG/Parent
C57MG/Wnt-1
Stra6 / GAPDH
3.5
3
2.5
2
1.5
1
0.5
0
DMSO
VD3
ATRA
9cRA
DMSO
VD3
ATRA
9cRA
B
C57MG/Parent
Stra6 / GAPDH
18
15
12
9
6
3
0
No treatment
Control media
Wnt-3A media
9cRA
Control media + 9cRA
Wnt-3A media + 9cRA
C
HCT116
Stra6 / GAPDH
0.3
0.2
0.1
0
DMSO
VD3
ATRA
9cRA
WiDr
Stra6 / GAPDH
2.5
2
1.5
1
0.5
0
DMSO
VD3
ATRA
9cRA
D
HCT116 -RA
HCT116 +RA
E
WiDr
-RA
+RA
F
-RA
+RA

FIGURE 18

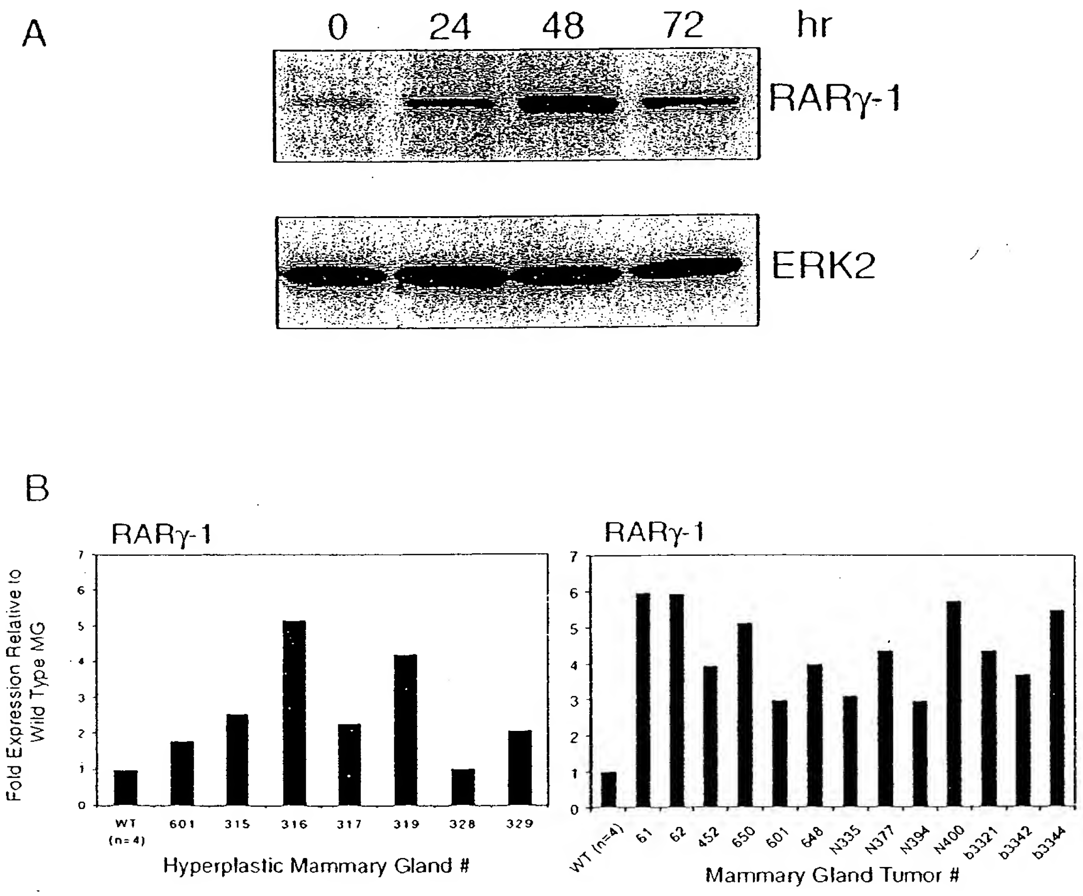

A
0
24
48
72
hr
RARγ-1
ERK2
B
RARγ-1
Fold Expression Relative to Wild Type MG
0
1
2
3
4
5
6
7
WT (n=4)
601
315
316
317
319
328
329
Hyperplastic Mammary Gland #
RARγ-1
0
1
2
3
4
5
6
7
WT (n=4)
61
62
452
650
601
648
N335
N377
N394
N400
b3321
b3342
b3344
Mammary Gland Tumor #

Figure 19

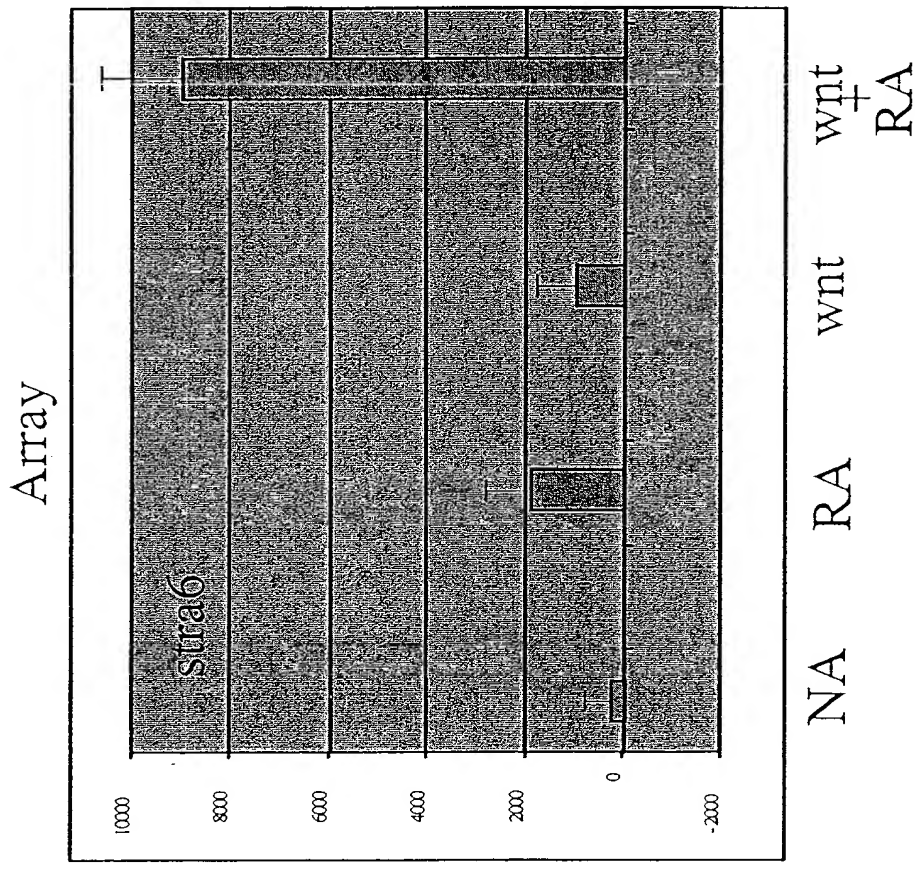
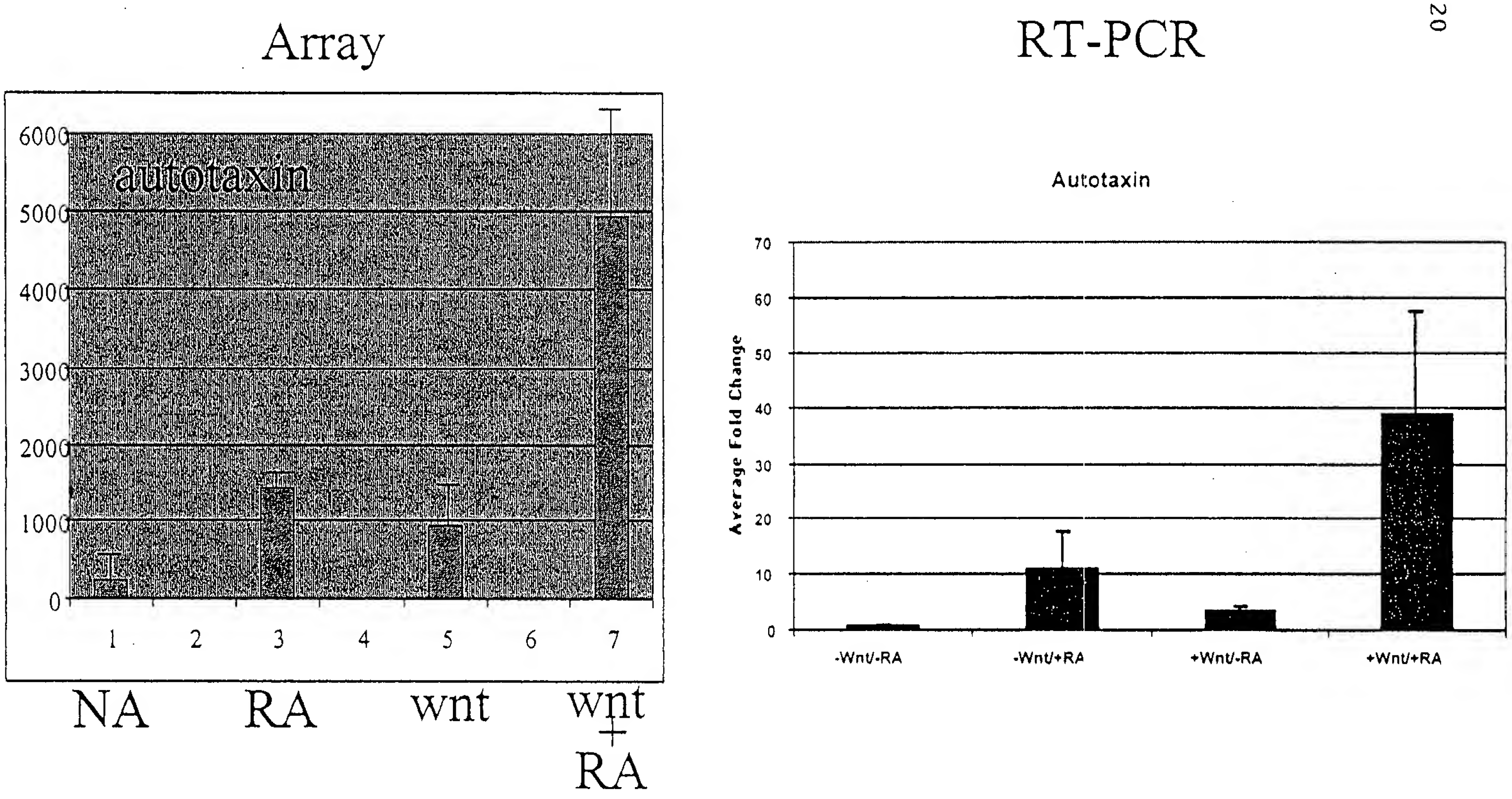
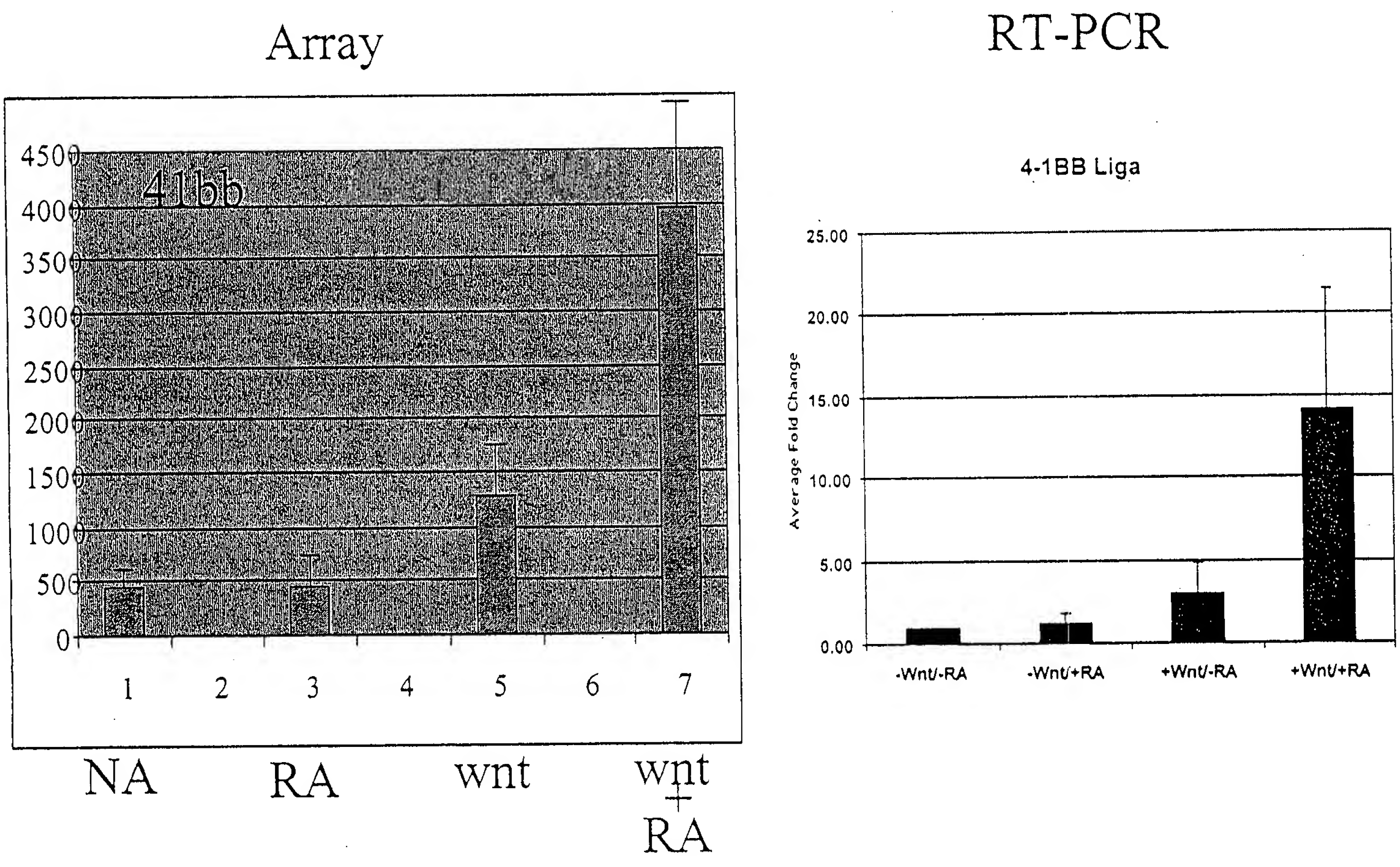


Figure 20


Array
autotaxin
6000
5000
4000
3000
2000
1000
0
1
2
3
4
5
6
7
NA
RA
wnt
wnt
+
RA
RT-PCR
Autotaxin
Average Fold Change
70
60
50
40
30
20
10
0
-Wnt/-RA
-Wnt/+RA
+Wnt/-RA
+Wnt/+RA

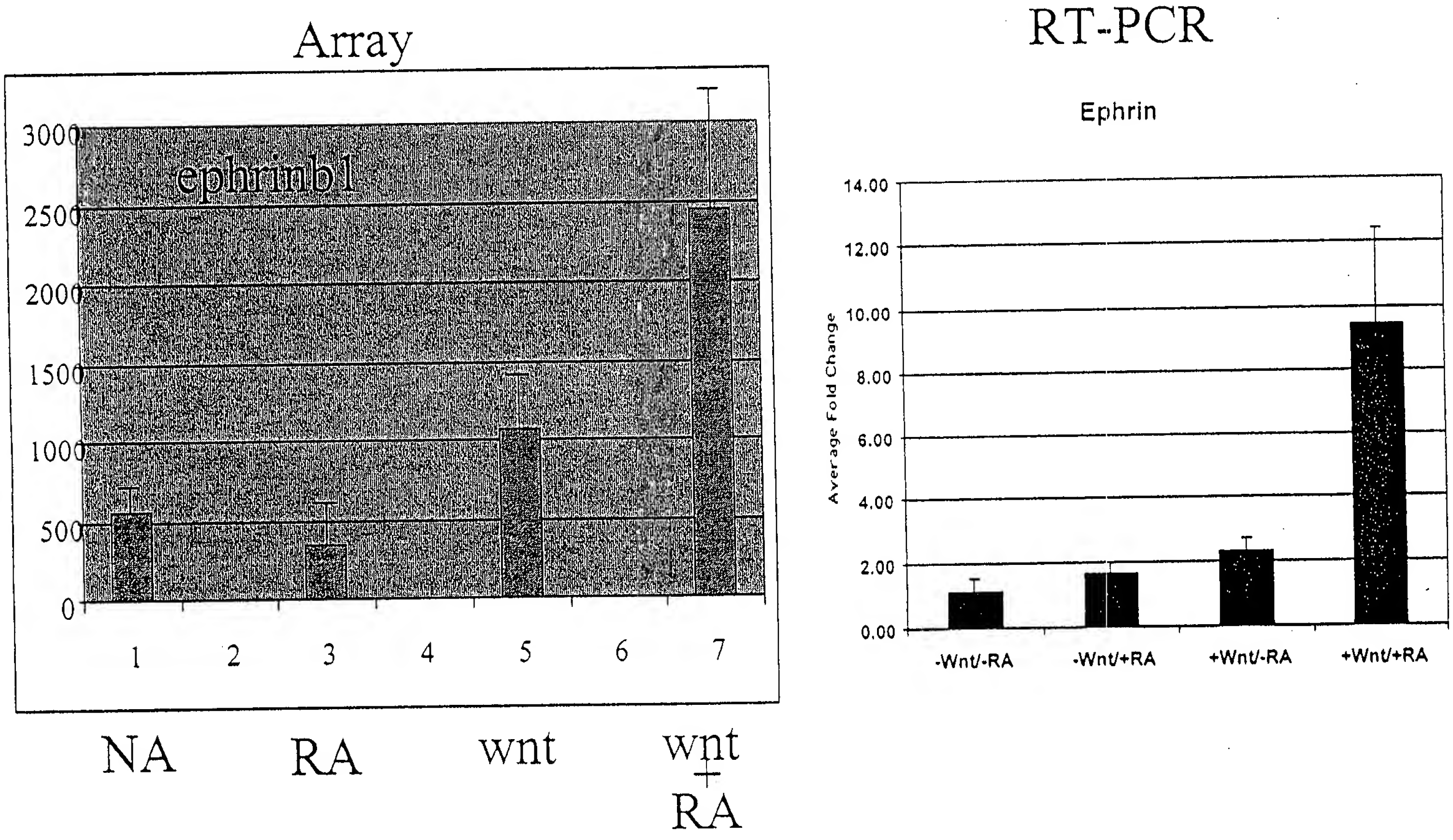


Array

RT-PCR

Figure 21

Figure 22



Array

RT-PCR

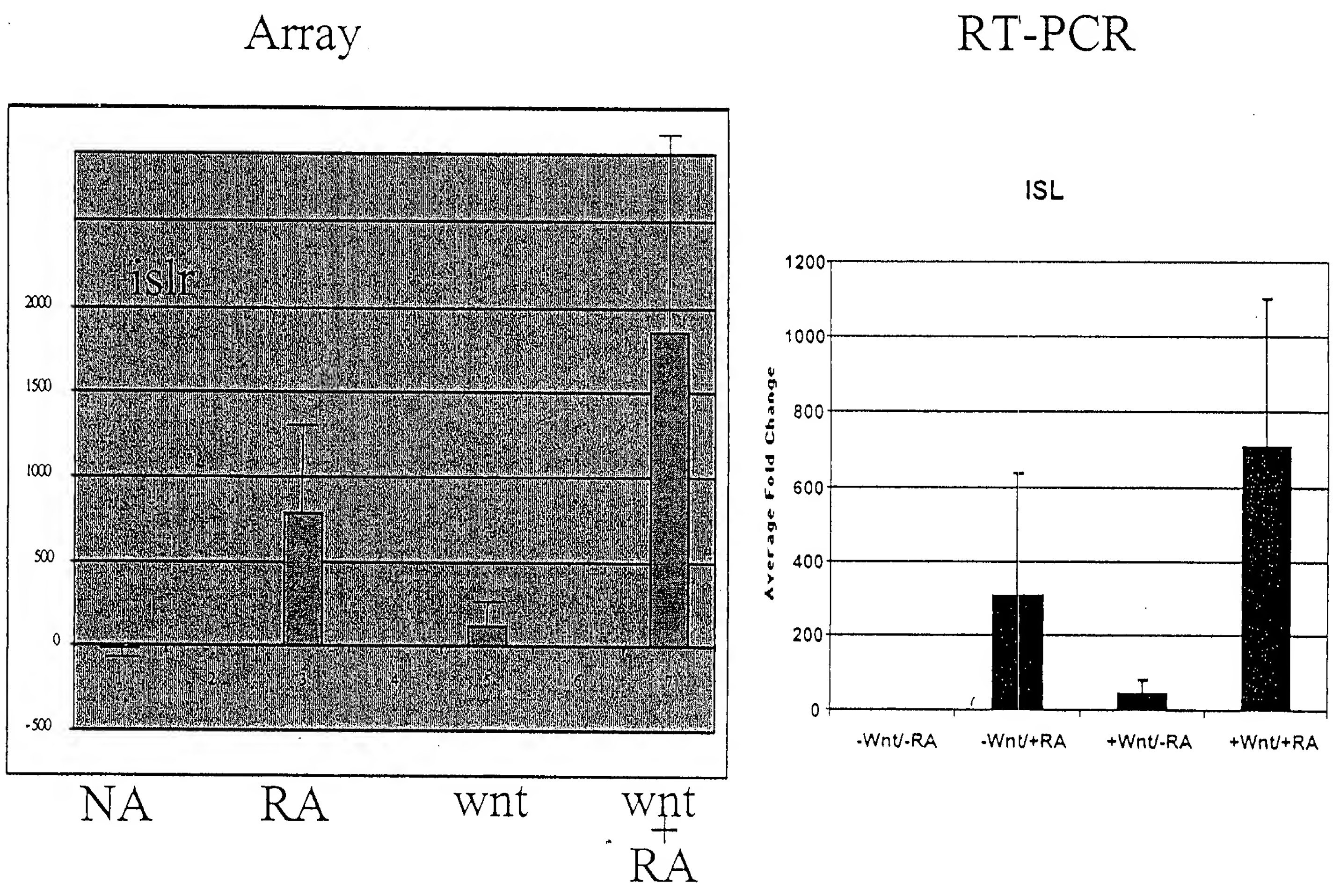

Array
islr
2000
1500
1000
500
0
-500
1
2
3
4
5
6
7
NA
RA
wnt
wnt + RA
RT-PCR
ISL
Average Fold Change
1200
1000
800
600
400
200
0
-Wnt/-RA
-Wnt/+RA
+Wnt/-RA
+Wnt/+RA

Figure 23

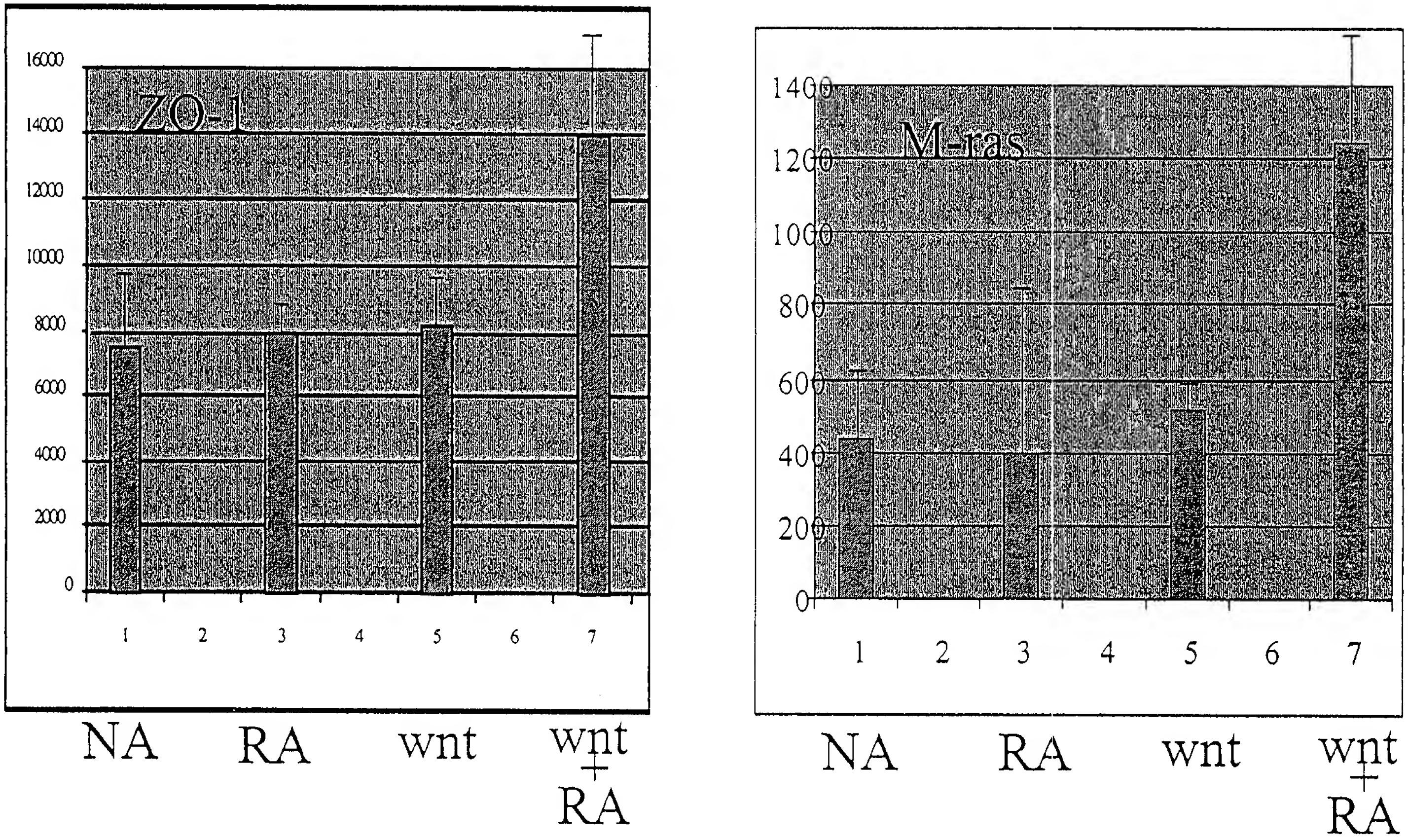

ZO-1
16000
14000
12000
10000
8000
6000
4000
2000
0
1
2
3
4
5
6
7
NA
RA
wnt
wnt
+
RA
M-ras
1400
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
NA
RA
wnt
wnt
+
RA

Figure 24

Figure 25

Figure 25 A

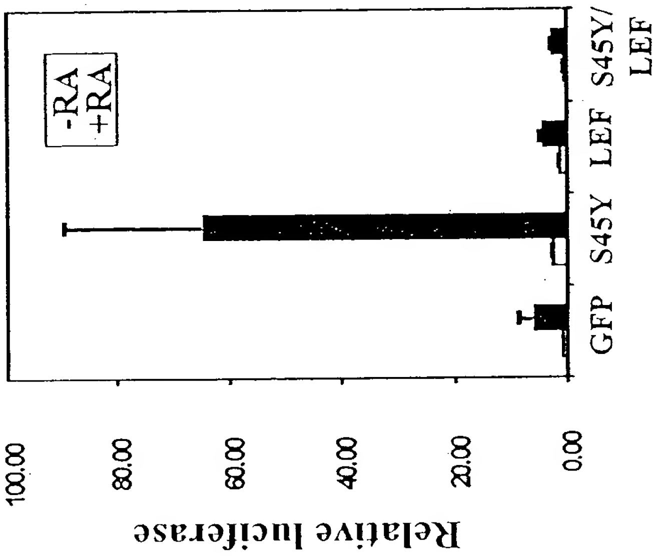


Figure 25 B

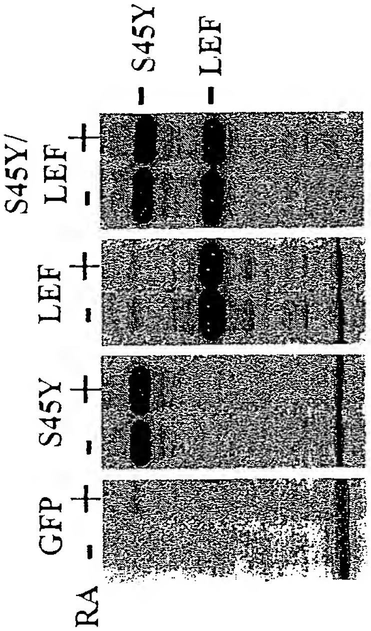
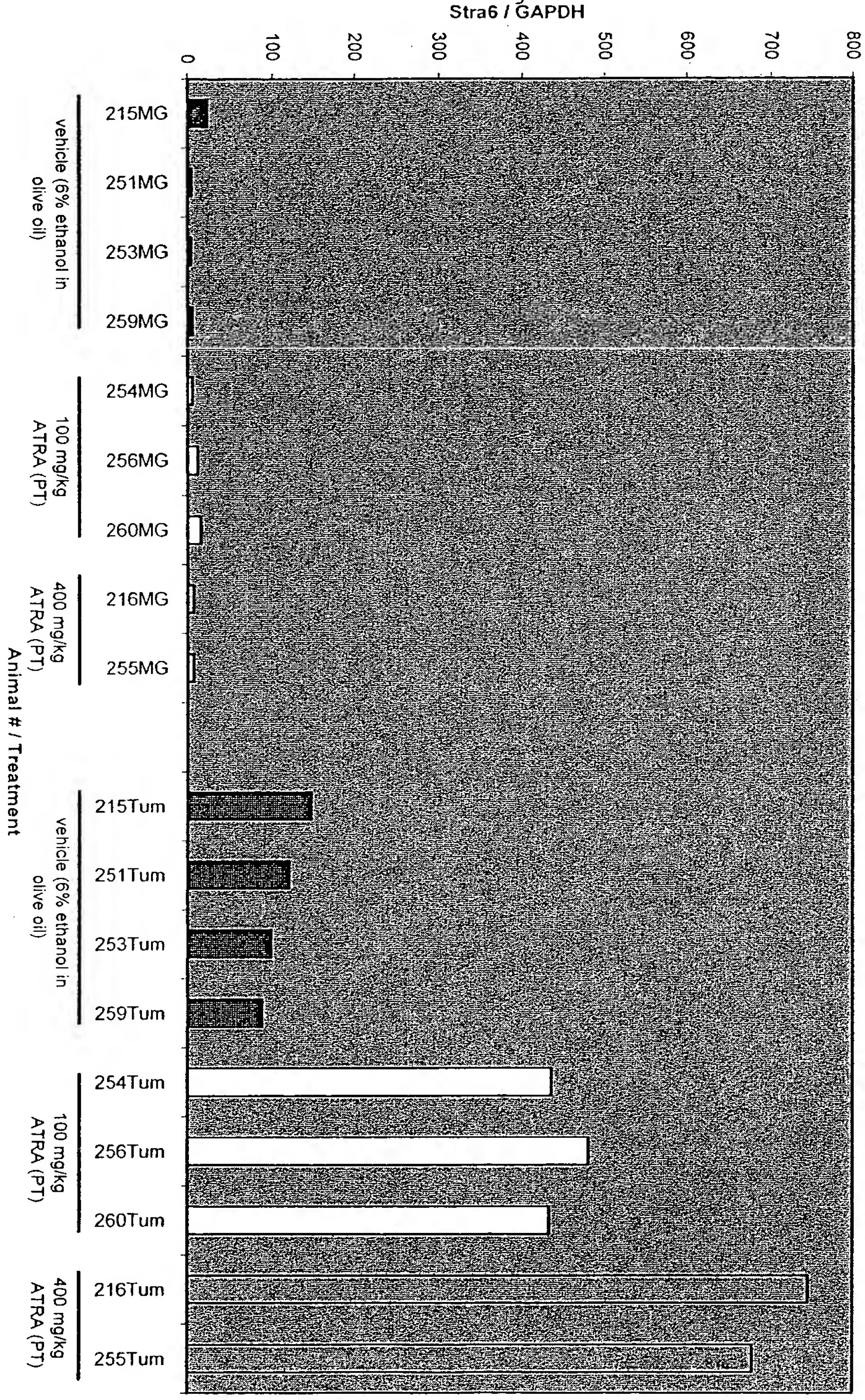


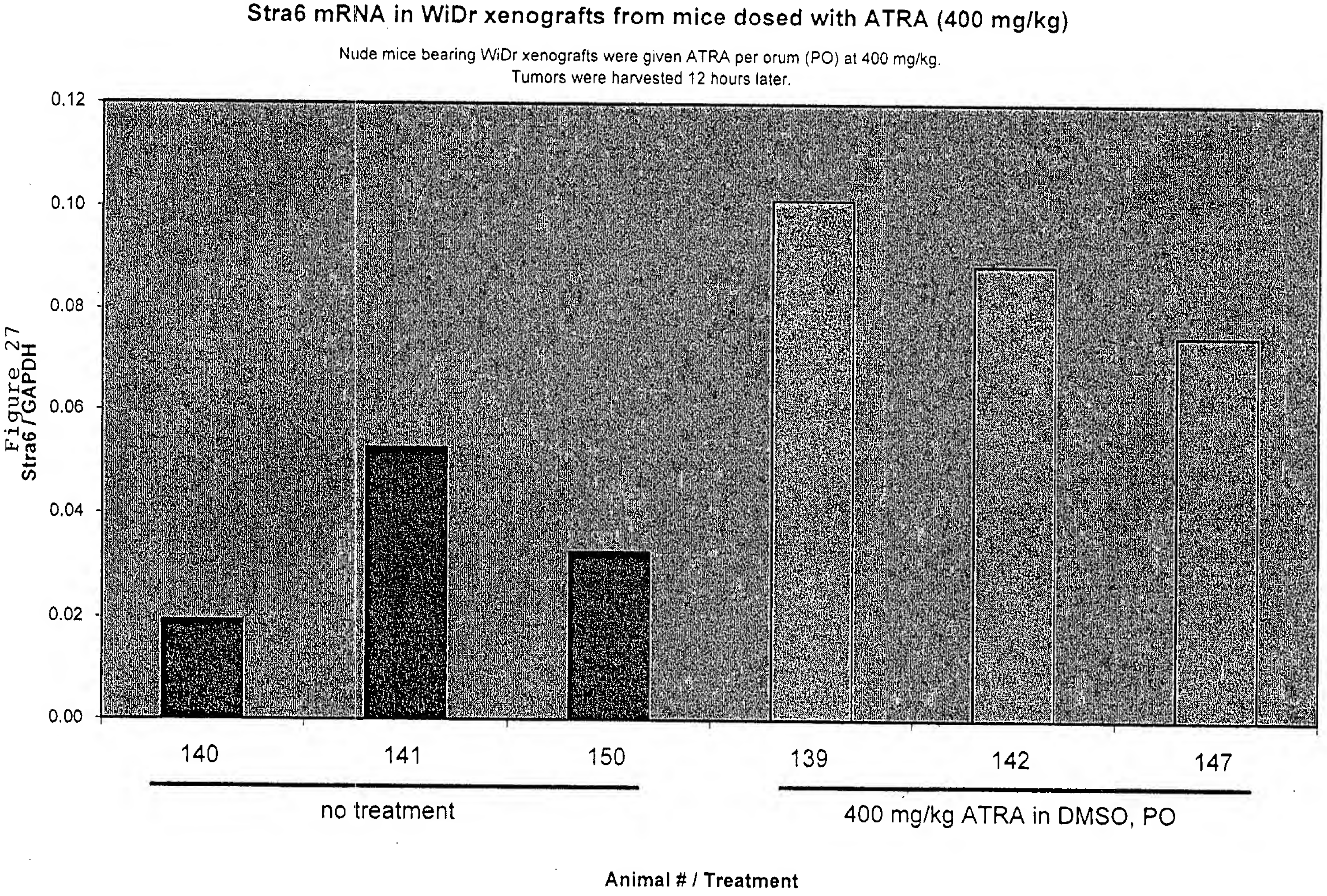
Figure 26



Stra6 mRNA in normal mouse mammary gland and Wnt-1mammary gland tumors

Nude mice bearing Wnt-1 tumor explants were given ATRA peri-tumorally (PT) at 100 and 400 mg/kg.
Tumors and normal adjacent mammary glands were harvested 8 hours later.

Figure 27


Stra6 mRNA in WiDr xenografts from mice dosed with ATRA (400 mg/kg)
Nude mice bearing WiDr xenografts were given ATRA per orum (PO) at 400 mg/kg.
Tumors were harvested 12 hours later.
Stra6 / GAPDH
0.12
0.10
0.08
0.06
0.04
0.02
0.00
140
141
150
139
142
147
no treatment
400 mg/kg ATRA in DMSO, PO
Animal # / Treatment